University of Alberta

The organic matter and microbial community composition of forest floors from boreal mixedwood stands in northern Alberta

by

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Abstract

Prior studies indicate that, in the boreal mixedwood forests of North America, forest floors that develop in stands dominated by trembling aspen (ASPEN; *Populus tremuloides*) tend to have a higher pH, and support more rapid rates of nutrient cycling and greater concentrations of microbial biomass than those that develop in stands dominated by white spruce (SPRUCE; *Picea glauca*). This pattern may be due to differences between stand types in soil microclimate, inputs from above- and belowground, the soil decomposer community and/or stand age. Using cross polarization, magic-angle spinning ^{13}C nuclear magnetic resonance spectroscopy, small but significant differences in organic matter composition were detected between ASPEN and SPRUCE forest floors; these differences appear to be due to stand type-specific humification pathways. The microbial community structure, assessed using substrate-induced respiration and phospholipid fatty acid analyses, of forest floors from SPRUCE and mixed-species (MIXED) stands were also very different than those from ASPEN stands; forest floor microbial community composition may be strongly affected by the presence of white spruce or, perhaps, the understory vegetation associated with white spruce. However, attempts to determine whether differences in inputs from belowground or in microclimate and inputs from aboveground were more important in controlling forest floor characteristics were largely unsuccessful, probably because of the short study time and the carbon-rich status of the forest floors. Although differences in organic matter composition were detected in the forest floors of ASPEN and SPRUCE clearcut and uncut stands, parallel differences in microbial community composition were not detected. The lack of a change in forest floor microbial community structure following harvesting

suggests that these communities are somewhat resilient to disturbance. This resilience may be the result of the careful harvesting techniques used in the study area. Future research should compare the influence of wildfire and timber harvesting on forest floor characteristics, investigate the effects of harvesting on organic matter decay patterns in these forest floors and more closely examine how forest floors from MIXED stands compare with those from ASPEN or SPRUCE stands.

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Chapter 1 Introduction

1.1 The boreal mixedwood forest

The boreal mixedwood forest of North America runs in a broad diagonal band from northeastern British Columbia to western Quebec. This forest is really a mosaic of stand types, whose structure and composition is driven largely by time since stand-initiating fire (Rowe, 1961; Dix and Swan, 1971), although other factors, such as the availability of seed and germination sites after disturbance, can also play a role (Purdy et al., 2002; Peters et al., 2005). In general, trembling aspen (*Populus tremuloides*) dominates the canopy of early seral stands; white spruce (*Picea glauca*) becomes more abundant with increasing stand age (Rowe, 1961; Viereck et al., 1983; Strong and Leggat, 1992).

The forest floor, which includes the surface organic horizons that overlay the mineral soil (Green et al., 1993), accounts for 10 to 35% of the total carbon found in the boreal mixedwood forest (Morrison et al., 1993; Huang and Schoenau, 1996; Nalder and Wein, 1999; Lee et al., 2002) and contains a significant portion of the site nutrient capital (Van Cleve et al., 1983; Bormann and Sidle, 1990; Prescott et al., 2000*b*). The forest floor that develops beneath a trembling aspen (ASPEN) canopy is quite different than that which develops under a white spruce (SPRUCE) canopy. Forest floors under aspen tend to have a higher pH, and frequently support more rapid rates of nutrient cycling, and higher concentrations of microbial biomass than forest floors under white spruce (Flanagan and Van Cleve, 1983; Coté et al., 2000; Lindo and Visser, 2003), although this is not always the case (Brais et al., 1995).

There are a number of possible explanations for the greater microbial activity frequently reported in ASPEN soils. These include more favourable microclimatic conditions and differences in organic matter quality. In the Alaskan taiga, for example, soil temperatures at 10 cm depth tend to be higher in ASPEN stands than in SPRUCE stands (Fox and Van Cleve, 1983), suggesting that forest floor microbial activity in SPRUCE stands may be hampered by cooler temperatures. However, SPRUCE forest floors tend to exhibit slower basal respiration rates than ASPEN forest floors even under identical conditions of moisture and temperature (Flanagan and Van Cleve, 1983; Lindo

and Visser, 2003). Thus, organic matter quality may be a more important factor controlling microbial activity in these forest floors than soil microclimate.

The higher organic matter quality of ASPEN forest floors is widely attributed to the reputed greater nutrient content, pH and decomposability of trembling aspen leaf litter compared with white spruce needles (Flanagan and Van Cleve, 1983; Lindo and Visser, 2003; Coté et al., 2000). However, broadleaf litter appears to decompose more rapidly than coniferous litter only in the initial stages of degradation (Prescott et al., 2000a; Prescott et al., 2004). Fine root exudates and fine root turnover also have the potential to strongly affect forest floor microbial activity and humus degradation (Finér et al., 1997; Coté et al., 2000; Grayston et al., 1996). Indeed, ASPEN forest floors have been reported to support a higher fine root biomass than SPRUCE forest floors (Finér et al., 1997; Lindo and Visser, 2003), and respiration rates in boreal stands appear to be more strongly related to the allocation of C to roots, than to litterfall (Ruess et al., 1996). Furthermore, the biomass of fine roots tends to correlate well with the abundance of mesofauna in the forest floors of boreal mixedwood stands (Lindo and Visser, 2003); differences in the activity of mesofauna have been proposed as yet another possible reason for the greater rates of litter decomposition sometimes observed in deciduous forests compared with coniferous forests (Prescott et al., 2000a). Thus, it remains unclear why the concentrations of available nutrients and levels of microbial biomass are typically higher in ASPEN forest floors than in SPRUCE forest floors, but inputs from above- and belowground, as well as processing by soil fauna, may play important roles.

Few studies have specifically compared the characteristics of forest floors from mixed-species (MIXED) stands (where neither trembling aspen nor white spruce dominate the canopy) to those of ASPEN and SPRUCE stands. This question is particularly interesting because MIXED stands are widely believed to exhibit greater productivity than those which are dominated by white spruce or by trembling aspen (Man and Lieffers, 1999; MacPherson et al., 2001; Rothe and Binkley, 2001). Recently, MIXED forest floors were found to support higher rates of nitrogen mineralization than forest floors from either ASPEN or SPRUCE stands (Jerabkova et al., 2006). However, most measures of N, phosphorus and carbon availability tend to be intermediate in

MIXED forest floors between those in ASPEN and SPRUCE forest floors (Kishchuk, 2002; Jerabkova et al., 2006).

1.2 Timber harvesting in the boreal mixedwood forest

Boreal mixedwood forests in North America are under increasing pressure from the timber and oil industries (Schneider et al., 2003), yet relatively little is known about the effects of timber removal on the long-term site productivity and integrity of these systems (Spence, 2001). Furthermore, there exists a growing interest in forest harvesting techniques that more closely emulate the effects of natural disturbance (e.g., Seymour and Hunter, 1998; Parminter, 1999; Kimmins, 2003). It is assumed that when the structure of the residual stand is similar after harvesting to that following natural disturbance, and includes such elements as standing and fallen trees, coarse woody debris and patches of undisturbed forest, the ecological processes occurring in that forest will also be preserved. However, monitoring and scientific study is required to justify and refine techniques, such as variable retention harvesting, which have been proposed to achieve these goals (Spence, 2001; Bergeron et al., 2004). The Ecosystem Management Emulating Natural Disturbance (EMEND) experiment was established to examine ecological patterns and processes in stands of varying tree species composition and to compare the effects of a range of levels of variable retention harvesting in order to provide scientifically-based guidance to the forestry industry for the protection of the ecological integrity of the boreal mixedwood forest. As such, harvesting treatments at EMEND include, among others, uncut controls, partial harvests in which 50% of the original stand was retained, partial harvests in which 20% of the original stand was retained, and clearcuts.

Forest floor characteristics may change following harvesting in response to alterations in soil moisture and temperature regimes, and shifts in the quantity, quality and timing of detrital inputs (Keenan and Kimmins, 1993; Prescott et al., 2000*b*; Ballard, 2000). Basal respiration rates were reduced in the forest floor of ASPEN stands two years after clearcutting in eastern Ontario (Weber, 1990), and in the forest floors of both SPRUCE and ASPEN stands 2.5 years after clearcutting at EMEND (Lindo and Visser, 2003). Reduced rates of respiration suggest that harvesting may lower the availability of labile C in these forest floors. Such a pattern is consistent with the suggestion that

harvesting induces changes in the nature of the forest floor, rendering it less capable of supporting a large microbial community (Hart et al., 1994; Bradley et al., 2000; Prescott et al., 2003). However, previous attempts to identify alterations in the organic matter composition of boreal soils following harvesting have been rare and any changes detected were subtle (Bååth et al., 1995).

The composition of the soil microbial community in different forest types may influence post-disturbance rates of nutrient cycling and, ultimately, regeneration success because soils with distinct patterns in microbial community structure also frequently exhibit differences in nutrient dynamics (Priha et al., 1999; Priha et al., 2001; Leckie et al., 2004; Grayston and Prescott, 2005). Therefore, it is critical to understand how the effects of clearcutting and partial retention harvesting influence the soil microbial community in stands of varying tree species composition. Compared with 1.5 year-old clearcuts, selection and gap-felling treatments successfully eliminated post-harvest reductions in the microbial biomass and respiration rates of soils from Norway spruce forests in Finland, but did not prevent significant changes in microbial community structure (Siira-Pietikäinen et al., 2001a). Two and a half years post-harvest at EMEND, forest floors from partial-cut SPRUCE stands exhibited similar concentrations of microbial biomass and rates of basal respiration to those from uncut controls; in contrast, forest floors from partial-cut ASPEN stands exhibited similar concentrations of microbial biomass and rates of basal respiration to those from clearcuts (Lindo and Visser, 2003). In southwestern Québec, Canada, the ratio of microbial C/N was reduced in soils from clearcuts but not in soils from partial-cut stands dominated by trembling aspen (Brais et al., 2004). Taken together, the results of these studies suggest that post-harvest changes in microbial biomass and microbial community structure may vary with stand composition. Further work is required to compare the effects of clearcutting and partial harvesting on the forest soil microbial communities and organic matter compositions of different stand types.

In summary, the boreal mixedwood forest includes ASPEN, mixed-species and SPRUCE stands. Previous studies have shown that the forest floors that develop in these stands exhibit different patterns of nutrient cycling and varying concentrations of microbial biomass. Variability in soil microclimate, litter inputs from above- and

belowground and soil biota have all been proposed as important factors influencing the characteristics of these forest floors. Changes in the soil organic matter and microbial community characteristics of forest floors following timber harvesting are hypothesized to result from altered soil microclimate and shifts in the quantity, quality and timing of inputs from above- and belowground. However, the effects of harvesting on the forest floor have not been extensively studied in the boreal mixedwood forest. Given the interest in forest management techniques that retain some portion of the original stand, further research is also required to examine the influence of clearcutting and variable retention harvesting in stands of differing tree species composition.

1.3 Objectives and outline

The overall objectives of this project were:

1. To determine how the organic matter composition of forest floors differed between stand types in the boreal mixedwood forest and to examine post-harvest changes in the organic matter composition of these forest floors

2. To determine how the microbial community composition of forest floors differed between stand types in the boreal mixedwood forest and to examine post-harvest changes in the microbial community composition of these forest floors

3. To examine the influence of above- and belowground inputs and soil microclimate on the microbial community structure and selected chemical characteristics of these forest floors.

This thesis is organized into six chapters. Following this introduction, Chapter 2 presents the findings of a study comparing the organic matter composition of FH-layer forest floors from undisturbed ASPEN and SPRUCE stands at EMEND. Because the techniques employed were time-consuming and expensive, MIXED stands were excluded from the study. Chapter 3 describes the changes in organic matter composition that occurred in FH-layer forest floors at EMEND 3.5 and 4.5 years after clearcutting. Again, because the techniques employed were time-consuming and expensive, undisturbed and clearcut ASPEN and SPRUCE stands were selected, to represent the most extreme effects of harvesting and stand type on organic matter properties. Chapter 4 presents the results of a study examining the microbial community composition in samples of forest floors from ASPEN, MIXED and SPRUCE stands at EMEND and describes how these

communities were affected by clearcutting and partial retention harvesting 4.5 and 5.5 years post-harvest. Chapter 5 describes the results of a reciprocal transfer study, using mesh bags that allowed or excluded fine root in-growth, in an attempt to isolate the effects of different factors on the composition of the forest floor microbial community in ASPEN and SPRUCE stands. Finally, Chapter 6 presents a summary of the previous four chapters, provides some suggestions for future research and discusses the implications of these results for forest management.

Tables and Figures

Table 1-1. List of abbreviations

Abbreviation	Definition
ACID	Proximate fraction extractable in sulfuric acid
AIR	Acid-insoluble residue fraction of proximate analysis
ALK	Carbon in the alkyl region of NMR spectra
ANOVA	Analysis of variance
AROM	Carbon in the aromatic region of NMR spectra
ASPEN	Stand dominated by deciduous trees, particularly trembling aspen
CARB	Carbon in the carbonyl region of NMR spectra
CHLORO	Proximate fraction extractable in chloroform
CLPP	Community-level physiological profiles, assessed using Biolog®
CPMAS	Cross-polarization and magic-angle spinning
DD	Dipolar-dephased
EMEND	Ecosystem Management Emulating Natural Disturbance
FAME	Fatty acid methyl esters
METH	Proximate fraction extractable in methanol
MIXED	Mixed stand of deciduous and coniferous trees
MRPP	Multiple response permutations procedure
NMR	Nuclear magnetic resonance
NMS	Non-metric multidimensional scaling
O-ALK	Carbon in the O-alkyl region of NMR spectra
PLFA	Phospholipid fatty acid
RISA	Ribosomal intergenic spacer analysis
SIR	Substrate-induced respiration
SOC	Soluble organic carbon
SOM	Soil organic matter
SON	Soluble organic nitrogen
SPRUCE	Stand dominated by coniferous trees, particularly white spruce
TMS	Tetramethylsilane
TPPM	Two-pulse phase modulation
WATER	Proximate fraction extractable in hot water

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Chapter 2 Forest floor composition in trembling aspen- and white spruce-dominated stands

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Introduction

High-resolution solid-state cross-polarization magic-angle spinning (CPMAS) ^{13}C nuclear magnetic resonance (NMR) spectroscopy is an exciting analytical tool for the characterization of soil organic matter (SOM) chemistry because, unlike more traditional techniques, it permits the direct study of whole-soil samples or SOM fractions without prior treatment (Knicker and Ludeman, 1995; Faz Cano et al., 2002). The potential of CPMAS ^{13}C NMR to detect differences in forest soil chemistry among vegetation communities has been demonstrated in numerous studies, and observed differences in soil chemistry have been attributed to a variety of soil-forming factors including climate, vegetation and landscape position. For example, de Montigny et al. (1993) hypothesized that differences in the composition of forest floors from stands dominated by western hemlock (*Tsuga heterophylla*) or western redcedar (*Thuja plicata*) on northern Vancouver Island, Canada, were caused by variability in soil moisture and the abundance of tannin-rich shrubs. In a comparison of soils from a grassland and a recently afforested site in New Zealand, Condron and Newman (1998) attributed higher O-alkyl carbon and lower alkyl carbon levels in the grassland soils to greater rates of litter input in the grassland than in the forest. By comparing a biosequence to an elevational transect, Quideau et al. (2001) concluded that vegetation, rather than climate, was controlling SOM composition. Under oak (*Quercus*), SOM was dominated by carbonyl C; under manzanita (*Arctostaphylos*), SOM was dominated by O-alkyl C, and under coniferous vegetation by alkyl C. Finally, Zech et al. (1989) reported that aromatic and alkyl carbon of uncultivated soils collected from Germany, Spain, and Liberia varied strongly with regional patterns of precipitation and temperature.

Studies such as these have been useful for detecting patterns in SOM chemistry among different ecosystems. However, because of time constraints and limited access to instrumentation, samples are typically composited prior to NMR analysis, thereby

obscuring within-sample variability, and precluding statistical analysis of treatment differences or correlations between NMR results and environmental variables. Only very rarely has statistical analysis of NMR results been specifically related to surrounding environmental conditions. In one of these studies, Faz Cano et al. (2002) suggested that statistical differences in O-alkyl and aromatic carbon in A-horizon soil among three vegetation communities and two climatic zones in Spain were due to variability in soil temperature. Preston et al. (2002) determined that forest floors from west-facing slopes on southern Vancouver Island, Canada, possessed more lignin features and fewer charcoal features than forest floors from east-facing slopes, which they hypothesized was the result of differences in the rates of blowdown and wildfire. Unfortunately, environmental variables were not measured in these studies, so the proposed explanations could not be verified. The use of statistical analysis to relate measured environmental characteristics, such as soil temperature, to NMR analyses could prove valuable in differentiating the effects of various soil-forming factors on SOM chemical composition.

The Ecosystem Management Emulating Natural Disturbance (EMEND) experiment, in northwestern Alberta, is a long-term research study covering 1000 ha of boreal mixedwood forest that includes stands dominated by trembling aspen (*Populus tremuloides* Michx.) or white spruce (*Picea glauca* (Moench) Voss). This controlled and replicated experiment was established, in part, to examine differences in ecosystem-level processes among stand types of the boreal mixedwood forest. A recent study of forest soils at EMEND revealed higher forest floor carbon contents (kg ha^{-1}), total C (%), and C:N ratios in white-spruce-dominated (SPRUCE) stands than in trembling-aspen-dominated (ASPEN) stands (Kishchuk 2002). Stand-type differences in the organic matter composition of the forest floor warrant further investigation because they may have implications for nutrient cycling processes and for the productivity of regenerating vegetation following logging. Indeed, the results of previous studies in the boreal mixedwood forest have suggested that forest floors in ASPEN stands are of higher quality (i.e., they contain more labile organic material) and support more rapid rates of nutrient cycling than forest floors in SPRUCE stands (Flanagan and Van Cleve, 1983; Paré and Bergeron, 1996; Ste-Marie and Paré, 1999; Coté et al., 2000).

The objectives of this study were:

1. to compare the chemical and local environmental characteristics (e.g., temperature, moisture, mass of surface materials) of forest floor material from stands dominated by white spruce or trembling aspen in the boreal mixedwood forest.
2. to explore relationships among measured chemical and environmental characteristics of these forest floors to gain insight into why these differences might occur.

Materials and Methods

Study site and sampling

Samples were collected in June 2002 from the EMEND experiment (56° 46' 13" N, 118° 22' 28" W). The EMEND site is located on the boreal mixedwood plain in the Upper Boreal-Cordilleran Ecoregion (Strong and Leggat, 1992). This ecoregion is characterized by cold winters (mean temperature -10.5 °C), warm summers (mean temperature 13.8 °C), and an average of 380 mm of precipitation, 2/3 of which usually falls during the summer (Strong and Leggat, 1992). The EMEND site is characterized by a rolling topography ranging in elevation from 677 to 880 m asl. Soils are usually Brunisols, Orthic Gray Luvisols or Dark Gray Luvisols developed on fine-textured glacio-lacustrine or glacial till parent materials (Kishchuk, 2004).

Samples were collected from three 10-ha replicates each of undisturbed SPRUCE and ASPEN stands (6 experimental units in total), which range in age from 70 to 130 years old. The SPRUCE stands consisted of > 70% white spruce, with some trembling aspen, balsam poplar (*Populus balsamifera*), paper birch (*Betula papyrifera*), balsam fir (*Abies balsamea*), or lodgepole pine (*Pinus contorta*). The understory of SPRUCE stands includes *Rosa acicularis*, *Sheperdia canadensis* and a dense ground cover of moss, especially *Hylocomium splendens, Pleurozium schreberi* and *Ptilium crista-castrensis*. Forest floors in SPRUCE stands are typically Humimors. Humimors are Mors whose profile is dominated by an H horizon that contains few recognizable plant residues (Green et al., 1993). Selected chemical data for the FH-layer of the forest floors in SPRUCE stands are shown in Table 2-1. The ASPEN stands consisted of > 70%

trembling aspen, with some of the tree species listed above for SPRUCE stands. The understory of ASPEN stands includes *Rosa acicularis*, *Viburnum edule*, and *Alnus* spp, with an herb layer of *Calamagrostis canadensis*, *Epilobium angustifolium* and *Cornus canadensis*. Forest floors in ASPEN stands are typically Mormoders. Mormoders are Moders whose diagnostic F-layer possesses evidence of both faunal activity and fungal hyphae; mormoders are considered an intergrade between moders and mors (Green et al., 1993). Selected chemical data for the FH-layer of the forest floors in ASPEN stands are provided in Table 2-1.

Six sampling sites were randomly selected within each experimental unit. At each sampling site, litter and moss were collected from within a 15 cm x 15 cm template that was placed on the forest floor surface. Recognizable leaves, needles, twigs, bark, seeds and cones were included as litter. This material was placed in plastic bags and kept on ice until it was transported to the laboratory where it was stored at ±5 °C for a maximum of 90 days. Below the litter and moss layers, the FH-layer of the forest floor within the 15 cm x 15 cm template was excavated to the depth of the mineral soil surface. All of the FH-layer forest floor material was placed in a plastic bag and kept on ice until it was transported to the laboratory, where it was stored at ±5 °C for a maximum of 30 days. The thickness of the FH-layer of the forest floor (from the surface of the F-layer to the surface of the mineral soil) was measured at the four corners of the 15 cm x 15 cm cavity. Immediately after sample collection, temperature measurements were taken 5 cm below the surface of the F-layer and 5 cm below the mineral soil surface using a temperature probe.

Sample preparation and NMR analyses

Samples of litter and moss were separated in the lab, dried at 65 °C for 48 hours and weighed. After the moss and litter materials were separated, the proportion of green moss in each moss sample was visually estimated to the nearest 5% and used to calculate the mass of green moss per sample. Samples of the FH-layer of the forest floor were sieved (6.3 mm) to remove roots and twigs, thoroughly mixed, and dried at 65 °C for 48 hours. Dried samples of the FH-layer material were finely ground using a ball mill. In order to compare the NMR spectra of the FH-layer forest floor with specific litter types, composite samples of white spruce needle litter, trembling aspen leaf litter (the most

common materials in the litterfall of SPRUCE and ASPEN stands, respectively; Lindo and Visser, 2003) and moss (*Hylocomium splendens*) were also dried at 65 °C for 48 hours and ground using a ball mill.

For two of the three replicates of SPRUCE and ASPEN stands, samples of the FH-layer of the forest floor collected from each of the six sampling sites within a replicate were composited prior to NMR analysis. For the remaining replicate of each stand type, NMR analysis was performed separately on the six subsamples collected within the replicate, in order to examine variability among samples.

Solid-state ^{13}C CPMAS NMR experiments were carried out on a Varian Chemagnetics CMX Infinity 200 (B_0=4.7 T, $\nu_L(^{13}C)$=50.3 MHz) NMR spectrometer using a 7.5-mm double-resonance MAS probe with high-power ^{1}H decoupling. All samples were packed into 7.5-mm (outer diameter) rotors with Zirconia (ZrO_2) sleeves, drive tips made of Kel-F, and end caps and spacers made of Teflon. All ^{13}C NMR spectra were acquired using cross-polarization (CP), and were referenced to TMS (δ_{iso}=0.0 ppm) by setting the high-frequency isotropic peak of solid adamantane to 38.56 ppm. (Earl and VanderHart, 1982; Bryce et al., 2001). The ^{1}H 90° pulse and Hartmann-Hahn matching conditions were also determined using this sample. All ^{13}C CP NMR spectra were acquired using a ^{1}H 90° pulse width of 4.5 μs, a pulse delay of 5.0 s, a contact time of 1.0 ms, an acquisition time of 17.1 ms, and a spinning frequency of 6.5 kHz. One thousand transients were collected for each sample of FH-layer forest floor material, litter, and moss that was analyzed. Two-pulse phase modulation (Bennett et al., 1995) with a ^{1}H decoupling field of 56 kHz was employed during the acquisition of all spectra. A Gaussian line broadening of 100 Hz was used to process all spectra. Contribution of the background signal to the spectra was determined by acquiring a spectrum of an empty rotor set under identical conditions as for the FH-layer material, litter, and moss samples. This contribution was subtracted from all Fourier-transformed ^{13}C NMR spectra before analysis.

Bruker's WIN-NMR package was used to estimate the relative integrated areas of various regions between 0 and 194 ppm. Many different spectral regions have been reported for the integration of ^{13}C NMR spectra (e.g., Skjemstad et al., 1997; Mao et al., 2000; Preston et al., 2000). In this study the spectral divisions were assigned based on

local minima of the spectra. The following regions were used for integration: 0~45 ppm, attributed to alkyl carbon (ALK); 45~112 ppm, attributed to O-alkyl carbon (O-ALK); 112~165 ppm, attributed to aromatic carbon (AROM), and 165~194 ppm, attributed to carbonyl carbon (CARB).

Dipolar-dephased (DD) spectra of FH-layer forest floor material from SPRUCE and ASPEN stands were produced by inserting a delay period of 40 μs (in the absence of ^{1}H decoupling) between the cross-polarization and the acquisition portions of the CPMAS pulse sequence (Hatcher, 1987). Peaks in spectra generated by DD correspond either to quaternary C or to C capable of some motion in the solid state (e.g., acetate or CH_2 in long chains; Lorenz et al., 2000). As a consequence, features of lignins and tannins can be more easily distinguished using DD than using CPMAS (Hatcher, 1987; Wilson and Hatcher, 1988; Lorenz et al., 2000). Peaks in CPMAS and DD spectra were compared to determine the relative importance of lignins and tannins in the FH-layer material from SPRUCE and ASPEN stands.

Statistical analyses

Data collected using solid-state CPMAS ^{13}C NMR experiments are considered 'semi-quantitative', primarily because of the variability in cross-polarization efficiencies and rates of relaxation among C atoms in different functional groups (Preston et al., 1997; Ussiri and Johnson, 2003; Smernik and Oades, 2003). As a result of this phenomenon, C in some chemical environments may be under-represented. This problem is of particular concern for C atoms that are not directly bonded to H atoms, such as those in highly condensed aromatic structures (Preston et al., 1997). However, the results of CPMAS ^{13}C NMR experiments are reproducible (Peuravuori et al., 2003). As a result, this technique can be used with confidence when the ultimate goal of the analyses is the comparison of trends and patterns among similar types of samples that have been analyzed under identical conditions (Kinchesh et al., 1995; Preston et al., 1997; Peuravuori et al., 2003), as was the case in our study.

The chemical and physical characteristics of the FH-layer forest floor material from SPRUCE and ASPEN stands were compared using analysis of variance for a completely randomized design. Prior to analysis, the temperatures of the FH-layer and mineral soil, and the moisture contents of the FH-layer were transformed (x^{-2}) to meet the assumptions

of normality and homogeneity of variance. Differences were considered statistically significant if $P<0.05$. Data transformations were not effective in obtaining a normal distribution for the mass of moss on the forest floor surface. Therefore, differences in the mass of moss from SPRUCE and ASPEN stands were compared using a Kruskal-Wallis nonparametric test after ranking the moss data (Zar, 1984). To examine relationships between the chemical and environmental characteristics of these forest floors, Pearson correlations were calculated across stand types (i.e., data from ASPEN and SPRUCE stands were analyzed together) and within stand types (i.e., data from ASPEN and SPRUCE stands were analyzed separately). Correlation analysis was performed only on the data for the 6 subsamples from one SPRUCE stand and 6 subsamples from one ASPEN stand that were not composited prior to NMR analysis. For the moss data, correlations were performed only on the data from the SPRUCE stand because there was no moss present at several sampling sites in the ASPEN stand. Correlations were considered statistically significant if $P<0.05$. Bonferroni corrections were used in the correlation analyses of data from SPRUCE and ASPEN stands to test for overall significance of the correlation matrices, which were considered not significant if the adjusted *P*-value was lower than the smallest *P*-value in the correlation matrix (Legendre and Legendre, 1998). All statistical analyses were performed using SAS (version 8.01, SAS Institute Inc. 1999-2000, Cary, N.C.).

Results

Forest floor chemical characteristics

Major signals in the spectra of FH-layer forest floors were found around 73 ppm, and were characteristic of the C-2, C-3, and C-5 carbons of cellulose and hemicelluloses (Figure 2-1). The shoulder at 63 ppm was assigned to the C-6 carbon in carbohydrates, while anomeric carbons were noticeable around 105 ppm (Teeaar and Lippmaa, 1984). In the ALK region of the spectra, the main peaks occurred around 30 ppm, suggesting that alkyl carbons present in the FH-layers of the forest floor in the two stands were mainly of the polymethylene type (Keeler and Maciel, 2000). The methoxyl carbon signal characteristic of lignins was apparent as a shoulder at 56 ppm in all spectra, although it was less well-resolved in spectra derived from the FH-layer forest floor of SPRUCE

stands. In the AROM region of the spectra, the small peaks at 130-131 ppm probably originated from C-substituted aromatic carbons, such as the C-1 carbon of guaiacyl and syringyl units, or the C-1, C-2 and C-6 carbons of *p*-hydroxyphenyl lignin moieties (Figure 2-1). The C-2 and C-6 carbons of syringyl lignin units likely contributed to the peak centered around 105 ppm (Preston et al., 2000), while the peak at 117 ppm may be derived from the C-2, C-5 and C-6 carbons of lignin guaiacyl units (Landucci et al., 1998; Preston et al., 2000). The C-3 carbons of guaiacyl units and the C-3 and C-5 carbons of syringyl units typically contribute a broad signal at 151-154 ppm (Landucci et al., 1998; Lorenz et al., 2000; Preston et al. 2000). This peak was apparent on all spectra, but the FH-layer material from SPRUCE stands showed an additional peak at 145 ppm, which was absent in the FH-layer material from ASPEN stands (Figure 2-1). Methoxylated C-3 carbons of the guaiacyl moieties were observed around 145-148 ppm (Landucci et al., 1998; Preston et al., 2000), although the occurrence of well-resolved maxima at 145 and around 154 ppm in the SPRUCE spectra are a characteristic marker for condensed tannins (Preston et al. 2000). In addition, tannins and tannin-like structures may have contributed to the signals at 105, 117 and 130 ppm. Finally, the peak at 175 ppm was indicative of the carbonyl carbon in acetyl and ester moieties (Skjemstad et al., 1997).

The distribution of peaks in the DD spectra confirmed that the content of condensed tannins is different in the FH-layer forest floors from SPRUCE and ASPEN stands. In DD spectra of FH-layer material from SPRUCE stands, the methoxyl signal of lignin, at 56 ppm, was not evident as a clear peak (Figure 2-1), while the strong peak around 130 ppm, and the presence of two peaks at 145 and 152 ppm are characteristic of materials with high tannin contents (Preston et al., 1997; Lorenz et al., 2000). In contrast, DD spectra of FH-layer forest floor material from ASPEN stands exhibited a clear peak at 56 ppm (Figure 2-1), consistent with a high lignin content. There was no strong peak at 130 ppm in DD spectra of FH-layer forest floor material from ASPEN stands, and only a single peak at 151 ppm. These observations indicate that lignins are relatively less abundant, and condensed tannins are relatively more abundant, in the FH-layer material from SPRUCE stands than from ASPEN stands (Preston et al., 1997; Lorenz et al., 2000).

The reproducibility of the NMR analyses was tested by performing the same analysis 6 times on a single sample on separate days, and integrating the resulting spectra (Table

2-2). The confidence intervals for the ALK, O-ALK, AROM and CARB regions of the 6 NMR spectra obtained from this one sample ranged from ±0.6 to ±0.9. These were small compared to the confidence intervals obtained from the analysis of 6 separate subsamples of FH-layer forest floor material from an ASPEN or SPRUCE stand, which ranged from ±0.9 to ±2.5 (Table 2-2). Therefore, differences in the NMR spectra of the FH-layer forest floors from the two stand types can be confidently assigned to differences in forest floor composition and not to random errors which might occur from the NMR analysis and integration of the spectra.

All ^{13}C NMR spectra were dominated by the O-ALK region, followed by the ALK, the AROM, and finally the CARB region (Figure 2-2). However, integration of the ^{13}C NMR spectra indicated differences in the composition of the FH-layer forest floor from SPRUCE and ASPEN stands. The FH-layer material from SPRUCE stands consisted of 21.4% ± 1.8% alkyl, 52.9% ± 2.1% O-alkyl, 17.4% ± 0.1% aromatic and 8.3% ± 0.4% carbonyl C while the FH-layer material from ASPEN stands consisted of 22.8% ± 1.6% alkyl, 52.1% ± 1.9% O-alkyl, 14.5% ± 1.5% aromatic and 10.6% ± 0.4% carbonyl C. Aromatic C was significantly more abundant in the FH-layer forest floors of SPRUCE stands than in those of ASPEN stands (P=0.027). Carbonyl C was significantly more abundant in the FH-layer forest floors of ASPEN stands than in those of SPRUCE stands (P=0.002).

Surface material chemical characteristics

As with the FH-layer forest floor, the greatest amount of organic C in white spruce needle litter, trembling aspen leaf litter or step moss was detected in the O-ALK region (Figure 2-3). The smallest amount of organic C in white spruce needle litter and trembling aspen leaf litter was detected in the CARB region. In contrast, the smallest amount of organic C in step moss was detected in the AROM region (Table 2-3). Lower levels of aromatic and carbonyl C and higher levels of O-alkyl C were found in white spruce needle litter, trembling aspen leaf litter and step moss than in the FH-layer forest floor material from either stand type. This was especially true for step moss, which consisted of more than 75% O-alkyl C and only 4% aromatic C.

In the ALK region, spectra from step moss and white spruce needle litter showed a peak around 20 ppm, characteristic of acetate CH_3 (Preston et al., 2000). Trembling aspen

leaf litter exhibited a peak around 33 ppm, which may be due to carbon in long chains of CH_2 that are more rigid than those resonating at 30 ppm (Lorenz et al., 2000). Peaks at 130-131 ppm and 151-157 ppm, associated with lignins and condensed tannins, were notably absent in the spectrum from step moss (Figure 2-3). The split peak at 169 and 174 ppm in the spectrum from white spruce needle litter may reflect the presence of cutins (Preston et al., 2000).

Relation to environmental variables

There were strong differences between SPRUCE and ASPEN stands in the quantity and type of materials on the forest floor surface, and in the characteristics of the FH-layer forest floor itself (Table 2-4). The mass of moss on the surface of the forest floor was greater in SPRUCE stands than in ASPEN stands (P=0.046), and the mass of the litter layer was greater in ASPEN stands than in SPRUCE stands (although differences were not statistically significant; P=0.057). The physical structure of the forest floors appeared to reflect these differences. The FH-layer of the forest floor from SPRUCE stands was thicker (P=0.009) with a lower bulk density (P=0.049) than that from ASPEN stands. This is consistent with the observation that decomposing moss, which is fluffy and fibrous, appeared to form the bulk of the forest floor in SPRUCE stands. On the other hand, there were no significant differences between SPRUCE and ASPEN stands in the moisture content or temperature of the FH-layer forest floor, or the temperature of the mineral soil (Table 2-4). However, two of the 18 sampling sites in SPRUCE stands were saturated and/or frozen about 20 cm below the forest floor surface at the time of sample collection; this was never observed in ASPEN stands. Furthermore, there was a trend toward lower mineral soil temperatures in SPRUCE than in ASPEN stands (P=0.095). However, it must be stressed that forest floor temperature and moisture was measured on only one date, at the time of forest floor sample collection.

Correlation analyses between forest floor chemical and environmental characteristics were performed both within and across SPRUCE and ASPEN stands (Table 2-5). Significant relationships between chemical and environmental variables were only found within stand types and not across stand types. The lack of significant correlations between the composition of the FH-layer forest floor and measured environmental characteristics across stand types suggests that environmental characteristics affect the

composition of the FH-layer forest floor differently in the two stand types. In SPRUCE stands, aromatic C in the FH-layer forest floor was negatively correlated with the mass (g m^{-2}) of green moss (P=0.030, although the overall correlation matrix for SPRUCE stands was not statistically significant). In ASPEN stands, alkyl C in the FH-layer forest floor was negatively correlated with the mass (g m^{-2}) of the litter layer (P=0.001), and O-alkyl C was positively correlated with mineral soil temperature (P=0.017).

Discussion

NMR analysis revealed statistically significant differences in the aromatic and carbonyl C contents of the FH-layer forest floors from SPRUCE and ASPEN stands. There are several factors that could have contributed to these differences, including (i) differences in the types and amounts of litter inputs to the forest floor, (ii) variations in the processes of decomposition and humification, as affected by litter chemistry, the presence/absence of certain groups of decomposer organisms, and environmental conditions (Baldock and Preston, 1995) or (iii) the age of the forest floor.

On the surface of the forest floor in SPRUCE stands, the mass of moss was double that of the litter layer, while in ASPEN stands it was about 10% that of the litter layer (Table 2-4). This is consistent with Rowe's (1956) observation that moss cover in the boreal mixedwood forest tends to increase with white spruce cover. Moss tissue is low in aromatic C because, as a non-vascular plant, it contains some phenols but no lignin or tannins (Williams et al., 1998). Thus, it is not surprising that within SPRUCE stands, FH-layer forest floors with high moss cover were associated with lower levels of aromatic C (Table 2-5).

The significantly higher levels of aromatic C in the FH-layer forest floor material from SPRUCE stands compared to that from ASPEN stands was surprising, given that moss was nearly ubiquitous in SPRUCE stands (Figure 2-2). Levels of aromatic C in white spruce needle litter were also lower than in the FH-layer forest floor of SPRUCE stands (Figure 2-2 and Table 2-3). Therefore, neither moss nor white spruce needle litter are a particularly rich source of aromatic C, and levels of aromatic C in moss and white spruce needle litter do not explain the higher levels of aromatic C in the FH-layer forest floor of SPRUCE stands compared to ASPEN stands.

In addition to litterfall, the contribution of belowground carbon inputs through fine root turnover must be considered (Zech et al., 1989). Strong and La Roi (1983) found that about 24% of white spruce biomass is allocated below ground. Unlike trembling aspen, whose lateral roots are typically concentrated at the forest floor:mineral soil interface, most white spruce lateral roots are found in the forest floor (Strong and La Roi, 1983). Therefore, fine roots probably contribute a larger amount of carbon to the FH-layer forest floors of SPRUCE stands than to ASPEN stands at EMEND. Fine roots were not analyzed in this study, but aromatic C in needles and fine roots of mature Norway spruce in southern Germany were similar to each other and to that found in white spruce needle litter in this study (11-13%; Rosenberg et al., 2003). Consequently, inputs of carbon from the fine roots of white spruce cannot fully explain the higher concentrations of aromatic C in the FH-layer forest floor of SPRUCE stands.

Woody litter inputs could also cause higher levels of aromatic C in the FH-layer forest floor of SPRUCE stands compared to that in ASPEN stands. However, the CPMAS and DD spectra of FH-layer forest floor material indicated that relatively more condensed tannins had accumulated in the forest floors of SPRUCE stands while relatively more lignin had accumulated in the forest floors of ASPEN stands (Figure 2-1). A buildup of condensed tannins in SPRUCE forest floor was unexpected because, unlike lignin, condensed tannins are not considered particularly resistant to degradation (Lorenz et al., 2000). Nonetheless, an accumulation of condensed tannins was also observed in the humus of a northern Ontario black spruce forest, and was hypothesized to be the result of environmental conditions that inhibited decomposition in the forest floor (Lorenz et al., 2000). Spruce stands in the Alaskan taiga tend to have higher soil moisture contents than ASPEN stands (Van Cleve and Powers, 1995). Differences in the temperature and moisture content of the FH-layer forest floors of SPRUCE and ASPEN stands at EMEND may not have been detected because measurements were taken only once, after several weeks of hot, dry weather. Therefore, the higher levels of aromatic C in the FH-layer forest floor of SPRUCE stands may be due to microclimatic conditions, such as high moisture contents, that hinder decomposition and favor the accumulation of condensed tannins in these forest floors.

Lower levels of carbonyl C in the FH-layer forest floor of SPRUCE stands than in ASPEN stands support the hypothesis that higher levels of aromatic C in the FH-layer forest floor of SPRUCE stands are the result of differences in the pattern of decomposition in these forest floors. Carbonyl C includes carboxylic acids, aldehydes and esters (i.e., relatively oxidized forms of carbon; Baldock and Preston, 1995). As organic material is aerobically decomposed, carbonyl C tends to increase (Kögel et al., 1987; Zech et al., 1987; Baldock and Preston, 1995). Therefore, lower carbonyl C levels in the FH-layer forest floor of SPRUCE stands than in ASPEN stands may be caused by the inhibition of oxidative degradation, perhaps during periods of saturation.

In ASPEN stands, alkyl C in the FH-layer forest floor was strongly negatively correlated with the mass of the litter layer (Table 2-5). This relationship was unexpected, because a greater mass of litter in ASPEN stands was anticipated to indicate greater inputs of alkyl C-rich trembling aspen leaves (Table 2-3 and Preston et al., 2000). Comminution and ingestion of litter by macrofauna, such as insects, cause minimal changes to the chemical composition of litter (Fox et al. 1994). Therefore, the inverse relationship between alkyl C in the FH-layer forest floor and the mass of surface litter in ASPEN stands may reflect: (i) faunal mixing of trembling aspen leaf litter into the forest floor, or (ii) reduction of trembling aspen foliage in the overhead canopy by defoliating insects, and its deposition, as frass, in the forest floor. Both possibilities merit further investigation.

In ASPEN stands, the significant positive relationship between O-alkyl C in the FH-layer forest floor and the temperature of the mineral soil contrasts with the results of a Spanish study, where O-alkyl C levels increased with decreasing soil temperature, presumably because SOM degradation was inhibited at low temperatures (Faz Cano et al., 2002). Although O-alkyl C levels generally decline with the degradation of litter material, there are some situations (e.g., more rapid production of O-alkyl C than alkyl or carbonyl C by soil microbes; Baldock et al., 1992), in which the relative abundance of O-alkyl C may increase with decomposition. Therefore, a positive relationship between mineral soil temperature and O-alkyl C levels in the FH-layer forest floor may still be consistent with enhanced microbial activity under higher soil temperatures, although this clearly requires further study.

The boreal mixedwood forest is a mosaic of stand ages and tree species whose structure and composition is believed to be driven by time since stand-initiating fire (Rowe, 1961). In northern Alberta, trembling aspen dominates the canopy of early seral mixedwood stands; with increasing stand age, white spruce becomes more abundant (Rowe 1961; Strong and Leggat, 1992). Therefore, the FH-layer forest floor of SPRUCE stands could differ from that of ASPEN stands simply because it has been allowed to develop for a longer period of time. Ratios of aromatic:O-alkyl C or alkyl:O-alkyl C are commonly used as indices of the extent of organic matter decomposition (Baldock and Preston, 1995). In the FH-layer forest floors of both SPRUCE and ASPEN stands, ratios of aromatic:O-alkyl C and alkyl:O-alkyl C were 0.3 and 0.4, respectively. The lack of a difference in either decomposition index between stand types suggests that differences in the composition of the FH-layer forest floors are the result of variability in humification pathways, rather than a factor of soil age. The importance of different humification pathways in distinguishing the FH-layer forest floors of the two stands types is supported by the correlation analyses. While these analyses showed significant correlations within stand types between the composition of the FH-layer forest floor and measured environmental characteristics, they revealed no clear relationships across stand types.

Conclusions

In summary, CPMAS ^{13}C NMR was used to characterize the FH-layer of forest floors from ASPEN and SPRUCE stands at EMEND. Aromatic carbon content was higher and carbonyl carbon content was lower in the forest floor of SPRUCE stands than in ASPEN stands. Within stand types, correlation analyses indicated significant relationships between the composition of the forest floor and soil temperature, the mass of the litter layer and the mass of the moss layer. However, these relationships could not explain observed differences in the chemical composition of the forest floor between stand types. Although forest floors from SPRUCE stands were largely composed of moss, which is low in aromatic C, they had a greater aromatic C content than forest floors from ASPEN stands, where moss was rare. Furthermore, a lack of significant correlations across stand types suggests that there are different relationships between the chemical and environmental characteristics of forest floors from SPRUCE and ASPEN stands.

Tables and Figures

Table 2-1. pH, total C, total N and C:N ratio of FH-layer forest floor material from a SPRUCE and an ASPEN stand.

	SPRUCE	ASPEN
pH	4.5 (0.3)	5.1 (0.3)
C (%)	46.0 (1.0)	41.1 (3.0)
N (%)	1.8 (0.1)	2.3 (0.1)
C:N	25.4 (1.4)	17.9 (0.6)

Note: Values are means with standard deviation in parentheses (n=3). pH was determined using a 1:10 ratio of forest floor to 0.01 M $CaCl_2$.

Table 2-2. Confidence intervals around ALK, O-ALK, AROM and CARB regions of ^{13}C NMR spectra obtained from (i) one sample of FH-layer forest floor material analyzed repeatedly (n=6), and (ii) uncomposited subsamples of FH-layer forest floor material collected from a SPRUCE and an ASPEN stand, and analyzed separately (n=6).

	ALK	O-ALK	AROM	CARB
	% of total peak area			
(i) One sample	17.4 ± 0.8	46.7 ± 0.9	24.1 ± 0.7	8.4 ± 0.6
(ii) Six subsamples				
SPRUCE stand	23.4 ± 2.5	50.7 ± 2.5	17.6 ± 1.6	8.3 ± 1.4
ASPEN stand	24.7 ± 2.2	50.4 ± 1.9	14.7 ± 1.2	14.7 ± 0.9

Note: Confidence intervals were calculated at $\alpha = 0.05$.

Table 2-3. Distribution of carbon in ALK, O-ALK, AROM and CARB regions of ^{13}C NMR spectra obtained from composite samples of trembling aspen leaf litter, white spruce needle litter and step moss collected from the forest floor surface.

Litter type	ALK	O-ALK	AROM	CARB
	% of total peak area			
White spruce needle litter	17.8	64.2	12.0	5.5
Trembling aspen leaf litter	26.0	59.9	8.5	5.9
Hylocomium splendens moss	12.1	78.2	4.1	5.6

Table 2-4. Differences in environmental characteristics associated with the FH-layer forest floor of SPRUCE and ASPEN stands.

	SPRUCE	ASPEN
Physical characteristics		
Mass of litter layer (g m^{-2})	0.16 (0.04)	0.30 (0.1)
Mass of moss (g m^{-2})	0.34 (0.04)	0.019 (0.03)*
Thickness of FH-layer forest floor (cm)	12.7 (2.3)	6.3 (0.4)***
Bulk density of FH-layer forest floor (Mg m^{-3})	0.060 (0.010)	0.080 (0.010)*
Microclimatic characteristics		
Moisture content of FH-layer forest floor (%)	190.3 (81.8)	195.1 (23.1)
Temperature of FH-layer forest floor (°C)	11.1 (0.8)	10.8 (0.9)
Temperature of mineral soil (°C)	4.6 (1.1)	8.6 (0.7)

Note: Values are means with standard deviation in parentheses (n=3). * indicates significance at the 0.05 probability level; *** indicates significance at the 0.001 probability level.

Table 2-5. Selected correlation coefficients (Pearson's *r*) describing the relationship between the chemical and environmental characteristics of the FH-layer forest floor in a SPRUCE and an ASPEN stand (n=6).

	ALK	O-ALK	AROM	CARB
		r		
SPRUCE stand				
Mass of green moss	0.71	-0.21	-0.85*	0.05
Mass of litter layer	0.78	-0.49	-0.47	-0.006
Mineral soil temperature	0.13	-0.32	0.034	0.33
ASPEN stand				
Mass of litter layer	-0.98***	0.69	0.68	0.05
Mineral soil temperature	-0.66	0.91*	0.30	-0.61

Note: * indicates significance at the 0.05 probability level; *** indicates significance at the 0.001 probability level. P-values were adjusted using Bonferroni corrections to test for overall significance of the correlation matrices: $P<0.004$ (SPRUCE) and $P<0.006$ (ASPEN).

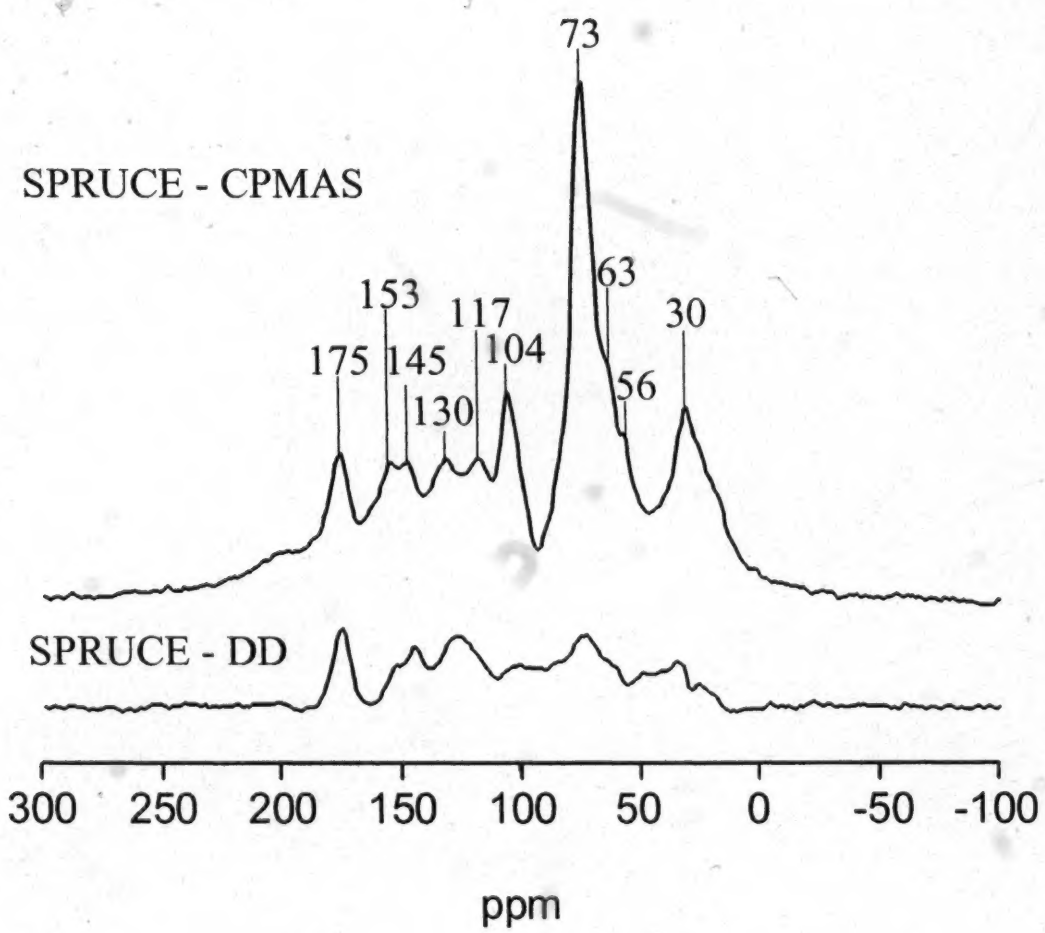

Figure 2-1. Representative cross-polarization magic-angle spinning (CPMAS) and dipolar-dephased (DD) ^{13}C NMR spectra of the FH-layer forest floor of a SPRUCE and an ASPEN stand.

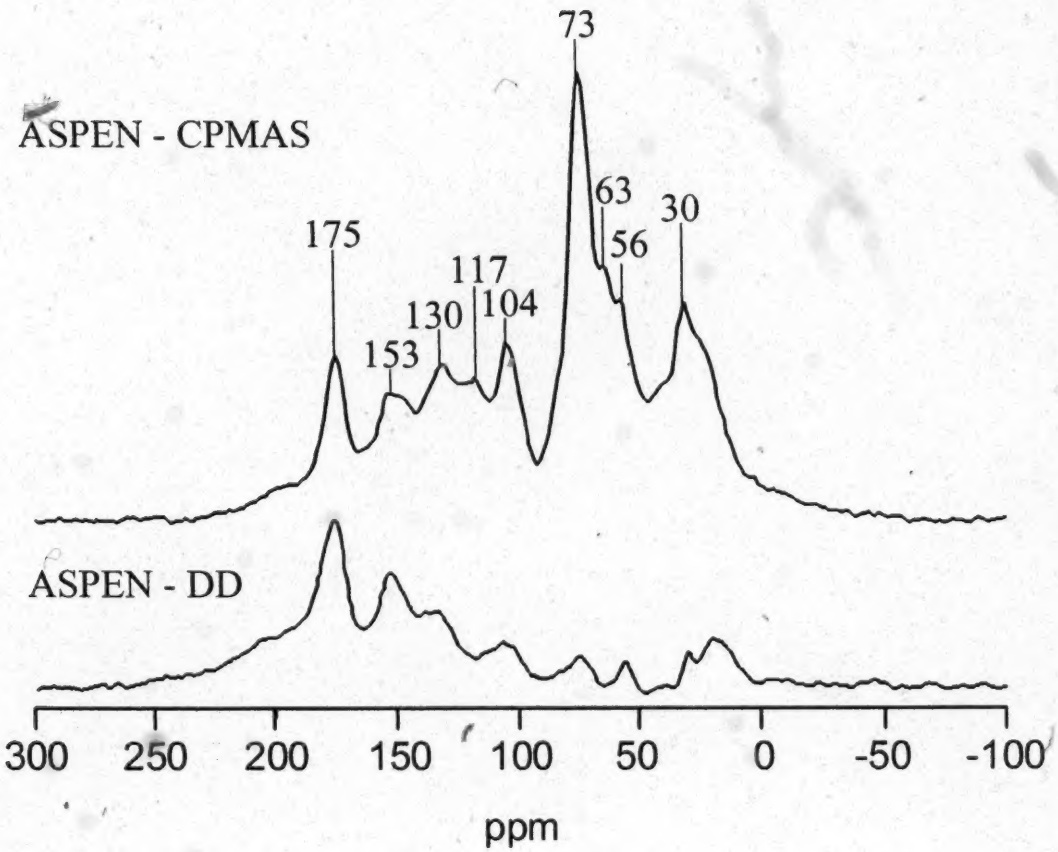

Figure 2-1 (continued).

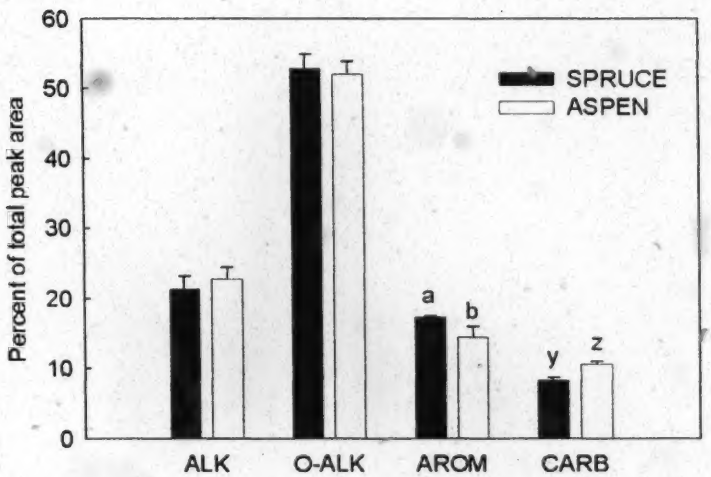

Figure 2-2. Distribution of carbon in the ALK, O-ALK, AROM and CARB regions of ^{13}C NMR spectra obtained from the FH-layer forest floor of SPRUCE and ASPEN stands.
Note: Error bars indicate one standard deviation (n=3). Different letters indicate significance at the 0.05 probability level.

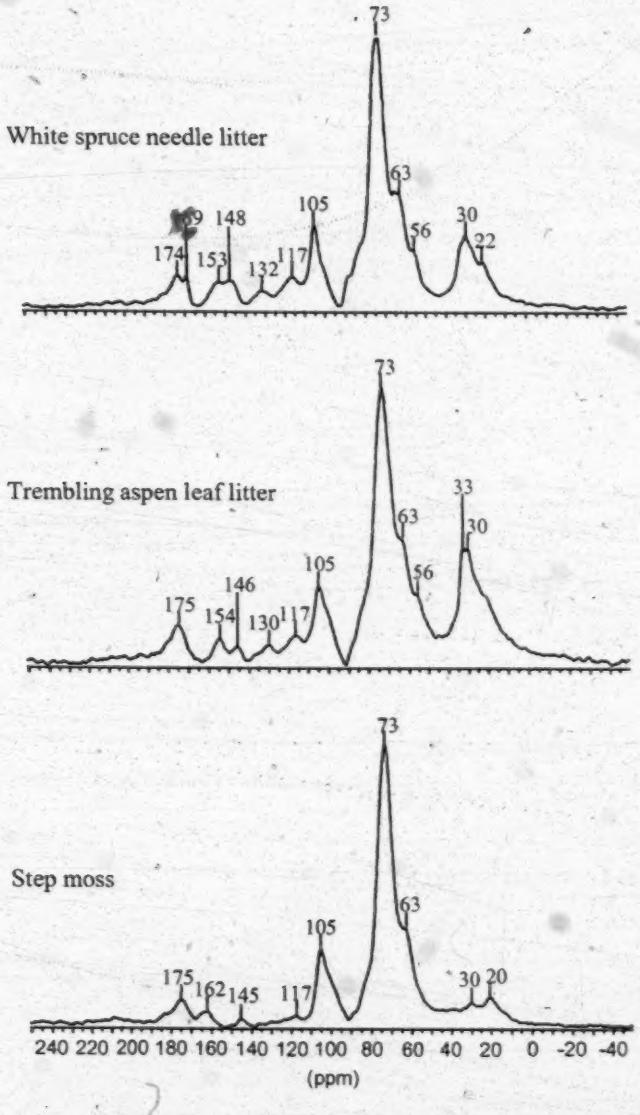

Figure 2-3. Representative ^{13}C NMR spectra of white spruce needle litter, trembling aspen leaf litter and step moss.

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Chapter 3 Forest floor chemical properties are altered by clearcutting in trembling aspen- and white spruce-dominated stands

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Introduction

Boreal mixedwood forests in North America are under increasing pressure from the timber and oil industries (Schneider et al., 2003), yet relatively little is known about the effects of timber removal on the long-term site productivity and integrity of these ecosystems (Spence, 2001). The forest floor, which includes the surface organic horizons that overlay the mineral soil (Green et al., 1993), accounts for 10 to 35% of the total carbon found in North American boreal forests (Morrison et al., 1993; Huang and Schoenau, 1996; Nalder and Wein, 1999; Lee et al., 2002) and contains a significant portion of the site nutrient capital (Van Cleve et al., 1983; Bormann and Sidle, 1990; Prescott et al., 2000*b*). Forest floor characteristics may change following clearcut harvesting in response to alterations in soil moisture and temperature regimes, and shifts in the quantity, quality and timing of detrital inputs (Keenan and Kimmins, 1993; Prescott et al., 2000*b*; Ballard, 2000). Given that organic matter quality controls the rate at which organic matter decomposes (Fogel and Cromack Jr., 1977; Melillo et al., 1982; Scott and Binkley, 1997), the size and composition of the microbial community that it supports (Vance and Chapin, 2001; Webster et al., 2001; Park et al., 2002) and the concentration of available nutrients (e.g., nitrogen) that it supplies (Stump and Binkley, 1993; Hart et al., 1994; Prescott et al., 2003), changes in forest floor organic matter properties could have implications for long- and short-term forest productivity (Prescott et al., 2000*b*; Dai et al., 2001). However, few studies have compared the properties of forest floor organic matter in clearcut and undisturbed forests (Dai et al., 2001; Preston et al., 2002).

A number of methods have been employed to assess organic matter properties in forest soils. These include separation of organic matter into broad chemical fractions using wet chemistry, i.e., proximate analysis (Fogel and Kromack Jr., 1977; McClaugherty et al.,

1985; Ryan et al., 1990), examination of changes in the ^{13}C isotopic composition of whole or fractionated organic matter (Nadelhoffer and Fry, 1988; Melillo et al., 1989; Quideau et al., 2003) and description of the chemical environment of organic C using cross-polarization magic-angle spinning ^{13}C nuclear magnetic resonance (CPMAS ^{13}C NMR) spectroscopy (Kögel et al., 1987; Zech et al., 1987; Baldock and Preston, 1995). All of these methods are complicated by a common problem - the forest floor is a complex material that is composed of substrates ranging from very labile to highly recalcitrant forms of organic matter (Berg and McClaugherty, 2003). Important changes in organic matter composition may not be detected because the most labile materials, and thus the most sensitive to change, are probably the least abundant (Dai et al., 2001; Preston et al., 2002). Therefore, a combination of techniques was used in the present study, with the aim of increasing sensitivity to changes in the properties of forest floor organic matter (Zech et al., 1992).

The Ecosystem Management Emulating Natural Disturbance (EMEND) experiment, in northwestern Alberta, is a long-term research study covering 1000 ha of boreal mixedwood forest that includes stands dominated by trembling aspen (*Populus tremuloides* Michx.) or white spruce (*Picea glauca* (Moench) Voss). This controlled and replicated experiment was established, in part, to examine differences among stand types in the response of various ecosystem-level processes to varying intensities of logging. The results of previous experiments suggest that forest floors from aspen-dominated (ASPEN) stands exhibit more rapid C, N and P cycling than forest floors from spruce-dominated (SPRUCE) stands (Flanagan and Van Cleve, 1983; Paré and Bergeron, 1996; Lindo and Visser, 2003). Following clearcutting, forest floors from both SPRUCE and ASPEN stands at EMEND exhibited less fine root biomass, lower quantities of litter input, reduced concentrations of microbial biomass C and decreased rates of C mineralization (Lindo and Visser, 2003). The aims of the present study were: i. to determine if the organic matter properties of forest floors from SPRUCE and ASPEN stands were altered by clearcutting, and ii. to determine whether post-harvest changes in the properties of forest floor organic matter were stronger in ASPEN or in SPRUCE stands.

Materials and Methods

Study site

The EMEND site (56° 46' 13" N, 118° 22' 28" W) is located on the boreal mixedwood plain in the Clear Hills Upland Ecoregion within the Boreal Plains EcoZone (Wiken, 1986; EcoRegions Working Group, 1989). The area is characterized by cold winters (mean temperature -14.0°C), warm summers (mean temperature 11.6°C), and an average 433 mm of precipitation, 2/3 of which usually falls during the summer (Environment Canada, 2004). The site is characterized by a rolling topography ranging in elevation from 677 to 880 m asl. Soils are usually Brunisols, Orthic Gray Luvisols or Dark Gray Luvisols that have developed on fine-textured glacio-lacustrine parent material (Kishchuk, 2004). Harvesting of the clearcuts, which are approximately 10 ha in size, was completed in the winter of 1998-1999. Whole trees were harvested using a feller-buncher and skidded directly to the landing, where stems were de-limbed. Debris from the de-limbing process was piled on the landing and burned (Sidders and Luchkow, 1998).

Sample collection

In June 2002, samples of forest floor were collected for assessment of bulk density, analysis using proximate fractionation, determination of ^{13}C isotopic composition and characterization using CPMAS ^{13}C NMR spectroscopy. Sampling for analysis using CPMAS ^{13}C NMR spectroscopy was repeated in June 2003. In both years, samples of forest floor were collected from three 10-ha replicates each of uncut (undisturbed) SPRUCE and ASPEN stands, which ranged in age from 70 to 130 years old, and three 10-ha replicates each of clearcut SPRUCE and ASPEN stands (12 experimental units in total). Uncut SPRUCE stands consist of > 70% white spruce, with some trembling aspen, balsam poplar (*Populus balsamifera*), paper birch (*Betula papyrifera*), balsam fir (*Abies balsamea*), or lodgepole pine (*Pinus contorta*). The understory of SPRUCE stands includes *Rosa acicularis*, *Sheperdia canadensis* and a dense ground cover of moss, especially *Hylocomium splendens, Pleurozium schreberi* and *Ptilium crista-castrensis*. Forest floors in SPRUCE stands are typically Humimors (Green et al., 1993). Selected descriptive data for the FH-layer forest floor of SPRUCE stands are provided in Table 3-1. Uncut ASPEN stands consist of > 70% trembling aspen, with some of the tree species

listed above for SPRUCE stands. The understory of ASPEN stands includes *Rosa acicularis*, *Viburnum edule*, and *Alnus* spp, with an herb layer of *Calamagrostis canadensis*, *Epilobium angustifolium* and *Cornus canadensis*. Forest floors in ASPEN stands are typically Mormoders (Green et al., 1993). Selected descriptive data for the FH-layer forest floor of ASPEN stands are provided in Table 3-1.

Six sampling sites were randomly selected within each experimental unit. Some prospective sampling sites were rejected in order to avoid visibly disturbed forest floors and rotten wood. Therefore, it should be noted that timber removal, rather than clearcutting *per se*, was the actual treatment effect being examined in this study. At each sampling site, the FH-layer forest floor within a 15 cm x 15 cm template that had been placed on the surface of the forest floor was excavated to the depth of the mineral soil surface. The F- and H-layers of the forest floor were not separated during sample collection because the F-layer was very thin in ASPEN stands and, therefore, difficult to separate from the H-layer. In SPRUCE stands, the bulk of the forest floor was dominated by decomposing moss, which made it difficult to distinguish the boundary between the F- and the H-layers. The thickness of the FH-layer forest floor (from the upper surface of the F layer to the surface of the mineral soil) was measured at the four corners of the 15 cm x 15 cm cavity. All of the FH-layer material removed from the cavity was placed in a plastic bag and kept on ice until it was transported to the laboratory, where it was stored at approximately 5 °C for a maximum of 30 days. Large roots and woody materials greater than 5 mm in diameter were removed, and each sample was weighed. Bulk density was calculated by dividing the dry mass of the whole sample (kilograms) by the volume of the sample (cubic metres, i.e., 0.15 m x 0.15 m x thickness of the FH-layer (m)).

For two of the three replicates of SPRUCE and ASPEN clearcuts and uncut stands, the six subsamples of FH-layer forest floor collected within each replicate in 2002 were composited prior to proximate fractionation, ^{13}C isotopic determination and ^{13}C NMR analyses. For the remaining replicate of each stand type x harvesting treatment combination, proximate fractionation, ^{13}C isotopic determination and ^{13}C NMR analyses were performed separately on the six subsamples collected within the replicate, to examine variability among subsamples and relationships among measured variables. All

six subsamples collected from each replicate in 2003 were composited prior to NMR analysis.

Proximate fractionation

Samples of FH-layer forest floor were sieved (6.3 mm) to remove roots and twigs, thoroughly mixed, and dried at 65 °C for 48 hours. Dried samples of the FH-layer forest floor were finely ground using a ball mill. An aliquot was heated at 500 °C overnight to determine the ash content. Non-polar extractives were determined using extraction in a 1:2 (v:v) mixture of chloroform and methanol (Bligh and Dyer, 1959; Suberkropp et al., 1976; Kögel-Knabner, 1995); water-soluble extractives were determined using hot water (100 °C) extraction (TAPPI, 1999); and acid-soluble and insoluble fractions were determined using a two-stage digestion in sulfuric acid (Effland, 1977). Non-polar extractives are more commonly estimated by extraction in dichloromethane (Heng and Goh, 1981; McClaugherty et al., 1985; Ryan et al., 1990) but a chloroform:methanol extractant, which includes a range of polarities, was chosen in order to produce higher yields of extract (Kögel-Knabner, 1995). However, it should be noted that the use of methanol in the non-polar extraction step might have removed some materials that are water-soluble. The fraction of chloroform- (CHLORO), methanol- (METH), water- (WATER) and acid-soluble (ACID) extractives, as well as acid-insoluble residues (AIR) were calculated on an ash-free basis (Ryan et al., 1990). Because water and methanol may have extracted similar materials, statistical analyses were also performed on the sum of the two fractions. The C and N contents and ^{13}C composition of finely ground unfractionated FH-layer forest floor, the WATER fraction (after freeze-drying), and the AIR were determined by combustion on a Costech C/N Elemental Analyzer interfaced using a Finnigan Mat Conflo III to a Finnigan Mat, Delta Plus Advantage Mass-Spectrometer. ^{13}C isotopic compositions were expressed as the ‰ deviation from the standard reference material, Pee Dee Belemnite.

CPMAS ^{13}C NMR analyses

Solid-state CPMAS ^{13}C NMR experiments were carried out on sieved, dried and ground forest floor samples using a Varian Chemagnetics CMX Infinity 200 (B_0=4.7 T, $\nu_L(^{13}C)$=50.3 MHz) NMR spectrometer using a 7.5 mm double-resonance MAS probe with high-power ^{1}H decoupling. All samples were packed into 7.5 mm (o.d.) Zirconia

(ZrO_2) sleeves, drive tips made of Kel-F, and end caps and spacers made of Teflon (Dupont, Circleville, OH). All ^{13}C NMR spectra were acquired using cross-polarization (CP), and were referenced to TMS (δ_{iso}=0.0 ppm) by setting the high-frequency isotropic peak of solid adamantane to 38.56 ppm. (Earl and VanderHart, 1982; Bryce et al., 2001). The ^{1}H 90° pulse and Hartmann-Hahn matching conditions were also determined using this sample. All ^{13}C NMR spectra were acquired using a ^{1}H 90° pulse width of 4.5 μs, a pulse delay of 5.0 s, a contact time of 1.0 ms (Preston et al., 1997), an acquisition time of 17.1 ms (Preston, 2001) and a spinning frequency of 6.5 kHz. One thousand transients were collected for each sample of forest floor material that was analyzed. A ^{1}H decoupling field of 56 kHz, using the two-pulse phase modulation (TPPM) scheme (Bennett et al., 1995), was employed during the acquisition of all spectra. A Gaussian line broadening of 100 Hz was used to process all spectra. The contribution of the background signal to the spectra was determined by acquiring a spectrum of an empty rotor set under identical conditions as for the forest floor material. This contribution was subtracted from all Fourier transformed ^{13}C NMR spectra before analysis. Spinning side bands were not corrected for because they did not appear to have a strong effect on any of the spectra.

Bruker's WIN-NMR package was used to estimate the relative integrated areas of various regions between 0 and 194 ppm. Many different spectral regions for the integration have been reported (e.g., Skjemstad et al., 1997; Mao et al., 2000; Preston et al., 2000). In this study the spectral divisions were assigned based on local minima of the spectra. The following regions were used for integration: ~0 to ~45 ppm attributed to alkyl carbon (ALK); ~45 to ~112 ppm attributed to O-alkyl carbon (O-ALK); ~112 to ~166 ppm attributed to aromatic carbon (AROM), and ~166 to ~194 ppm attributed to carbonyl carbon (CARB). AROM includes regions associated with both C-aryl C (~112~140 ppm) and O-aryl, or phenolic, C (~140~160 ppm).

Dipolar dephasing (DD) ^{13}C NMR spectra of FH-layer forest floor material from SPRUCE and ASPEN stands were produced by inserting a delay period of 40 μs (in the absence of ^{1}H decoupling) between the cross-polarization and the acquisition portions of the CPMAS pulse sequence (Hatcher, 1987). Peaks in spectra generated by DD probably correspond either to quaternary C or to C capable of significant motion in the solid state (e.g., methyl C; Lorenz et al., 2000). As a consequence, features of lignins and tannins

can be more easily distinguished using DD than using CPMAS pulse sequence (Hatcher, 1987; Wilson and Hatcher, 1988; Lorenz et al., 2000). Peaks in CPMAS and DD spectra were compared to determine the relative importance of lignins and tannins in the FH-layer forest floor material from uncut and clearcut SPRUCE and ASPEN stands.

Statistical analyses

Data collected using solid-state CPMAS ^{13}C NMR experiments are considered 'semi-quantitative', primarily because of variability in ^{1}H-^{13}C cross-polarization efficiencies (Preston et al., 1997; Ussiri and Johnson, 2003; Smernik and Oades, 2003). Thus, CPMAS ^{13}C NMR spectroscopy cannot be used to determine the quantities of different C types within a sample, but can be used to compare the relative abundance of different C types among similar samples, provided that they are analyzed under identical conditions (Kinchesh et al., 1995; Preston et al., 1997; Peuravuori et al., 2003).

Data obtained by proximate fractionation, and determination of ^{13}C isotopic composition were analysed using two-by-two (harvest x stand type) factorial ANOVA for a completely randomized design. Data obtained by CPMAS ^{13}C NMR spectroscopy were analysed using a two-by-two (harvest x stand type) factorial ANOVA for a completely randomized design with year as a split effect. When significant interactions were detected between stand type and harvesting treatments, one-way ANOVA was used to examine harvesting effects within stand types. Differences were considered statistically significant if $P<0.05$. To determine the relationships between the results of the proximate analyses, ^{13}C analyses and CPMAS ^{13}C NMR analyses, Pearson correlations were calculated using the data for the six subsamples that had been collected in June 2002 from a SPRUCE clearcut, SPRUCE uncut stand, ASPEN clearcut and ASPEN uncut stand and not composited prior to chemical analysis (Table 3-7). Correlations were considered statistically significant if $P<0.05$. Bonferonni corrections were used to test for overall significance of the correlation matrix (Legendre and Legendre, 1998). Data did not require transformation to meet the assumptions of the analyses. All statistical analyses were performed using SAS (version 8.01, SAS Institute Inc. 1999-2000, Cary, N.C.).

Results

Proximate fractionation

ACID and AIR were the most abundant fractions in FH-layer forest floors, together accounting for approximately 85% of the total forest floor mass (Table 3-2). WATER was the next most abundant fraction, making up about 10% of the total forest floor mass, with CHLORO and METH each accounting for less than 3%. Two-way ANOVA revealed no significant effects of clearcut harvesting or stand type on the distribution of organic matter among proximate fractions in FH-layer forest floors (data not shown).

Regardless of the source of the sample, unfractionated forest floors had greater concentrations of C (mg g^{-1}) and higher C:N ratios than the WATER fraction, and smaller concentrations of C and lower C:N ratios than the AIR fraction (Table 3-3). In addition, unfractionated forest floors were depleted in ^{13}C relative to the WATER fraction and enriched in ^{13}C relative to the AIR fraction. The C:N ratio and abundance of ^{13}C in unfractionated forest floor, in the WATER fraction and in the AIR fraction from SPRUCE stands were consistently greater than those from ASPEN stands (Table 3-4). Unfractionated FH-layer forest floor from SPRUCE stands also had a higher concentration of C than that from ASPEN stands. The ^{13}C composition of the AIR fraction showed a significant interaction between stand type and harvesting treatment. One way ANOVA indicated that the AIR fraction of FH-layer forest floors from clearcut ASPEN stands was significantly enriched in ^{13}C relative to FH-layer forest floors from uncut ASPEN stands (Table 3-3; P=0.049). In contrast, the ^{13}C composition of the AIR fraction of SPRUCE forest floors was not significantly different (at P<0.05) in clearcuts and uncut stands.

NMR spectroscopy

The major peaks in the ^{13}C NMR spectra of FH-layer forest floors from uncut SPRUCE and ASPEN stands, which have been described elsewhere (Hannam et al., 2004), were similar to those of FH-layer forest floors from clearcuts (Figure 3-1). Briefly, the peaks at 30 ppm in the ALK region indicate that alkyl C in these forest floors was mainly of polymethylene origin (Keeler and Maciel, 2000), while the shoulders at 56 ppm, which were stronger in FH-layer forest floors from clearcut and uncut ASPEN

stands, indicate the presence of methoxyl C from lignin. The O-ALK region was dominated by a peak at 73 ppm, with a shoulder at 63 ppm, characteristic of carbon in carbohydrates such as cellulose and hemicelluloses (Preston et al., 2000). In the AROM region, the small peaks at 104 ppm, 117 ppm and 130-131 ppm probably originate from aromatic C in guaiacyl, syringyl and *p*-hydroxyphenyl lignin monomers and from tannins or tannin-like structures (Preston et al., 2000). A single peak, probably originating from the methoxylated aromatic carbons of guaiacyl and syringyl lignin monomers, was apparent around 151-154 ppm in all spectra of FH-layer forest floor from ASPEN stands. Spectra of FH-layer forest floors from SPRUCE stands showed two peaks, one at 145-148 ppm and the other at 151-154 ppm, that are considered indicative of the presence of condensed tannins (Landucci et al., 1998; Lorenz et al., 2000; Preston et al., 2000). Finally, the CARB region was dominated by a peak at 175 ppm, indicative of the carbonyl carbon in acetyl and ester moieties (Skjemstad et al., 1997).

DD ^{13}C NMR spectra of FH-layer forest floors from SPRUCE stands showed a strong peak at 130 ppm and two overlapping peaks centered at 145 and 152, while DD ^{13}C NMR spectra of FH-layer forest floors from ASPEN stands showed a clear peak at 56 ppm and a single peak at 151 ppm. Such a pattern indicates that FH-layer forest floors from uncut and clearcut SPRUCE stands were relatively depleted in lignin and enriched in condensed tannins compared with FH-layer forest floors from uncut and clearcut ASPEN stands (Preston et al., 1997; Lorenz et al., 2000).

Qualitative differences in the NMR spectra of FH-layer forest floors from uncut and clearcut stands of either stand type were not immediately obvious, apart from a general broadening of peaks in the spectra of FH-layer forest floors from the clearcuts. However, the abundance of O-aryl C (~140~160 ppm) was relatively lower than that of C-aryl C (~112~140 ppm) in FH-layer forest floors from the clearcuts of both stand types, although the pattern was most obvious in the DD ^{13}C NMR spectra of forest floors from SPRUCE stands (Figure 3-1).

^{13}C NMR spectra of FH-layer forest floor samples collected in 2002 and 2003 from uncut and clearcut SPRUCE and ASPEN stands were dominated by the O-ALK region, followed by the ALK, the AROM, and finally the CARB region (Table 3-5). The year of sample collection had a strong effect on the distribution of C within all NMR regions

(Table 3-6). Samples collected in 2002 exhibited consistently higher relative concentrations of alkyl and O-alkyl C and consistently lower relative concentrations of aromatic and carbonyl C than samples collected in 2003. This pattern may reflect year-to-year differences in the moisture content (Table 3-1) and, thus, the degree of microbial processing of organic matter in the two years, or small differences in the type of forest floor material collected for analysis in 2002 and 2003. Nonetheless, there were no significant interactions between year and harvesting or between year and stand-type effects, indicating that the effects of harvesting and stand-type on the organic matter composition of these forest floors did not change with sampling date. Thus; O-ALK was significantly more abundant in FH-layer forest floors from SPRUCE stands, while CARB was significantly more abundant in FH-layer forest floors from ASPEN stands (Table 3-6). Furthermore, there were significant interactions between stand type and harvesting treatment in the abundance of AROM and in the ratio of AROM:OALK in these forest floors. One-way ANOVA indicated that the ratio of AROM:O-ALK, an index of the extent of organic matter decomposition (Baldock and Preston, 1995), was significantly greater in FH-layer forest floors from clearcut ASPEN stands than from uncut ASPEN stands in 2003 (P=0.019; Table 3-5). There was also a strong trend toward greater AROM in FH-layer forest floors from clearcut ASPEN stands than from uncut ASPEN stands in 2003 (P=0.071).

Correlations between proximate fractions, ^{13}C isotopic composition and NMR regions

There were a number of significant relationships between the results of proximate fractionation, determination of ^{13}C isotopic composition, and analysis using CPMAS ^{13}C NMR spectroscopy. For example, there was a negative relationship between ACID and CARB and between AIR and O-ALK, but a positive relationship between AIR and CARB (Table 3-7). The ^{13}C composition of the acid-insoluble residue (^{13}C-AIR) was negatively correlated with ALK and CARB and positively correlated with AROM and WATER+METH.

Discussion

Despite the relatively short time since harvest, there was considerable evidence to suggest that the organic matter composition of FH-layer forest floors from ASPEN and SPRUCE stands had been altered by timber removal: i. the acid-insoluble residue of forest floors from clearcut ASPEN stands was enriched in ^{13}C relative to the acid-insoluble residue of forest floors from uncut ASPEN stands, ii. aromatic C concentrations were higher in forest floors from clearcuts, particularly in ASPEN stands, and iii. phenolic C was lower in forest floors from clearcuts, particularly in SPRUCE stands. Such changes are probably related to reductions in above- and belowground inputs of fresh organic material after clearcut harvesting and to continued decomposition of the organic matter in the forest floors of these clearcut stands.

Following clearcutting, forest floors from both SPRUCE and ASPEN stands at EMEND exhibited less fine root biomass and lower quantities of litter input. However, fine root biomass and litter input rates remained higher in forest floors from ASPEN clearcuts than from SPRUCE clearcuts (Lindo and Visser, 2003). Given that dissolved organic C leached from senesced foliage and exudates from fine roots are important sources of microbially available C (Clein and Schimel, 1995; Grayston et al., 1996; Magill and Aber, 2000; Park et al., 2002), stronger quantitative changes in the composition of FH-layer forest floors from clearcut ASPEN stands than from clearcut SPRUCE stands may be due to differences in the rates of microbial activity and, thus, organic matter processing in these forest floors. Indeed, concentrations of microbial C and C mineralization rates were greater in forest floors from clearcut ASPEN stands than from clearcut SPRUCE stands 2.5 years after harvesting at EMEND (Lindo and Visser, 2003), suggesting that microbial activity was probably greater in the FH-layer of clearcut ASPEN stands.

Stand-type differences in the susceptibility of forest floor organic matter to decomposition may also have contributed to differences in the pattern of change observed in the forest floors from ASPEN and SPRUCE stands after clearcut harvesting. Although relative concentrations of O-ALK (associated with carbohydrates) were greater in FH-layer forest floors from SPRUCE stands than from ASPEN stands (Table 3-5), there was a stronger trend in ASPEN stands toward reduced O-ALK in forest floors from the

clearcuts, particularly in 2003 (P=0.10). Given that a large fraction of carbohydrates in forest soil may be physically protected and, therefore, less susceptible to decomposition (Zech et al., 1992), stronger treatment differences in the O-ALK of FH-layer forest floor from ASPEN stands suggests that a larger fraction of the carbohydrates in these forest floors was available for microbial degradation than that in SPRUCE forest floors. If this is the case, carbohydrates in FH-layer forest floors from ASPEN stands may have more strongly stimulated microbial decomposition of the AIR fraction, leading to a greater change in the ^{13}C composition of the AIR fraction in forest floors from ASPEN stands than from SPRUCE stands.

The increased abundance of ^{13}C in the AIR fraction of FH-layer forest floors from ASPEN stands was unexpected because the AIR is generally the fraction that is considered most resistant to microbial decay (McClaugherty et al., 1985; Melillo et al., 1989; Ryan et al., 1990). As a result, the AIR was not expected to show significant changes in composition in the short time that had elapsed since harvesting. Enrichment in ^{13}C during aerobic decomposition is widely believed to be caused by discrimination against ^{13}C during the catabolic breakdown of organic substrates by soil microbes and/or accumulation of ^{13}C in the microbial biomass and in humic materials of microbial origin (Melillo et al., 1989; Ehleringer et al., 2000; Quideau et al., 2003). This pattern may have been absent in SPRUCE forest floors because guiacyl monomers that are present in conifer lignin are more depleted in ^{13}C and less susceptible to mineralization than the syringyl monomers that dominate lignin of deciduous origin (Hedges et al., 1985; Goñi and Eglinton, 1996). Regardless of the specific mechanisms involved, the ^{13}C enrichment of the AIR fraction in ASPEN stands is probably due to the continued decomposition and humification of organic C in the FH-layer forest floors of these stands after clearcutting.

Decomposition and humification of organic matter has been associated with an increased abundance of aromatic C following clearcut harvesting in previous work using ^{13}C NMR. Higher aromatic C concentrations were detected in soil solution collected from a 14 year-old clearcut in New Hampshire compared with soil solution from an uncut hardwood stand nearby (Dai et al., 2001). However, no differences in aromatic C were detected in the organic matter composition of forest floor from different seral stages of coastal forest on Vancouver Island, which may have been due to the fact that these forest

floors were dominated by slowly-decomposing, woody material (Preston et al., 2002). In the present study, woody material was avoided during forest floor sampling, which may have made it easier to detect harvesting effects on forest floor organic matter characteristics, and aromatic C in particular.

Despite stronger quantitative differences in the relative abundance of AROM in FH-layer forest floors from clearcut and uncut ASPEN stands, there is evidence for more advanced microbial processing of aromatic C in forest floors from clearcuts than from uncut stands of both stand types. DD ^{13}C NMR spectra of forest floors from clearcut SPRUCE and ASPEN stands revealed a shift in the aromatic region toward a greater abundance of C-aryl C, indicating that less aromatic C in forest floors from the clearcuts had originated directly from plant material (Zech et al., 1992). Instead, this material had been more thoroughly processed and modified by forest floor microbes. In fact, the trend appeared to be stronger in forest floors from the SPRUCE clearcuts. Thus, quantitative changes in the organic matter composition of FH-layer forest floors were stronger in clearcut ASPEN stands, but important qualitative changes had also occurred in FH-layer forest floors of clearcut SPRUCE stands.

Although increased soil temperatures and moisture contents are widely believed to cause increased rates of decomposition following clearcut harvesting (Bormann et al., 1974), such an effect does not appear to be a contributing factor in this study. As mentioned above, Lindo and Visser (2003) found that microbial biomass and microbial respiration rates were reduced in forest floors from clearcuts relative to those from uncut stands of both stand types 2.5 years post-harvest. Furthermore, forest floor moisture contents were not strongly enhanced in the clearcuts of either stand type when samples were collected in 2002 and 2003, and a litter bag study revealed no evidence for more rapid decomposition in the clearcuts at EMEND (Lucie Jerabkova, The University of British Columbia, personal communication). Therefore, the changes in forest floor organic matter composition that occurred following clearcutting at EMEND do not appear to be the result of enhanced rates of decomposition.

Despite the fact that boreal forest floors have high ratios of C:N (Huang and Schoenau, 1996; Schwendenmann, 2000; Vance and Chapin, 2001; Ekblad and Nordgren, 2002), low organic matter quality is believed to limit the availability of C to

the forest floor microbial community (Flanagan and Van Cleve, 1983; Vance and Chapin, 2001; Ekblad and Nordgren, 2002). Our results suggest that timber removal altered the organic matter properties of forest floors from both SPRUCE and ASPEN stands at EMEND. In other forested ecosystems, post-harvest changes in the nature of the forest floor appear not only to lower the microbial activity of forest soils but also to modify nutrient cycling patterns (Hart et al., 1994; Bradley et al., 2000; Prescott et al., 2003). Data from previous studies at EMEND indicate that mineral N and P cycles in the forest floors of SPRUCE and ASPEN stands have been altered by clearcutting, but the pattern and timing of these changes appear to be stand type-specific, with forest floors from ASPEN stands generally responding more strongly and more rapidly than those from SPRUCE stands (Kishchuk, 2002; Lindo and Visser, 2003). Such a pattern is consistent with the observation that the organic matter of FH-layer forest floors from ASPEN stands is also more sensitive to clearcutting than that from SPRUCE stands. The relationship between forest floor organic matter properties and patterns of nutrient cycling remains unclear but is currently being examined at EMEND using pool dilution techniques and enzyme assays (Lucie Jerabkova, The University of British Columbia, personal communication).

Conclusions

In summary, 3.5 and 4.5 years after harvesting, the organic matter composition of FH-layer forest floors from clearcut ASPEN and SPRUCE stands was different than that from uncut stands. The AIR of FH-layer forest floors from ASPEN stands was significantly enriched in ^{13}C, probably as a result of microbial processing. Differences in organic matter composition, as revealed by CPMAS ^{13}C NMR spectroscopy, indicated that forest floors from clearcut SPRUCE and ASPEN stands had become more enriched in aromatic C. This trend was strongest in FH-layer forest floors from ASPEN stands. Furthermore, aromatic C in FH-layer forest floors from the clearcuts of both stand types showed a shift toward a greater abundance of C-aryl C, suggesting that these forest floors had become more humified. This pattern appeared to be stronger in forest floors from SPRUCE stands. Taken together, these results indicate that the organic matter of FH-layer forest floors from both clearcut SPRUCE and ASPEN stands has been modified, but changes appear to be strongest in ASPEN stands. Previous studies suggest a link between

post-harvest changes in the nature of forest soil organic matter and altered patterns of nutrient cycling, a hypothesis which is under investigation.

Tables and Figures

Table 3-1. Characteristics of FH-layer forest floors from uncut and clearcut SPRUCE and ASPEN stands.

	SPRUCE		ASPEN	
	Uncut	Clearcut	Uncut	Clearcut
Moisture content (%)				
- 2002	203.3 (66.9)	217.4 (38.8)	195.1 (23.1)	184.4 (34.1)
- 2003	301.8 (55.7)	307.3 (32.8)	254.4 (35.6)	238.8 (19.3)
Thickness (m)	0.14 (0.02)	0.10 (0.01)	0.08 (0.01)	0.10 (0.02)
Bulk density($Mg\ m^{-3}$)	0.060 (0.010)	0.069 (0.018)	0.080 (0.010)	0.095 (0.012)
Ash ($g\ kg^{-1}$)	122.5 (24.2)	144.1 (14.7)	183.9 (5.6)	187.6 (53.6)
pH	4.5 (0.3)	4.8 (0.5)	5.1 (0.3)	5.6 (0.5)

Note: Each value is the mean of three replicates with standard deviation in parentheses. pH was determined using a 1:10 ratio of forest floor (fresh weight in grams) to 0.01 mol L^{-1} $CaCl_2$ (milliliters).

Table 3-2. Mean concentration ($mg\ g^{-1}$ ash-free basis) of proximate fractions in FH-layer forest floors from uncut and clearcut SPRUCE and ASPEN stands.

	SPRUCE		ASPEN	
	Uncut	Clearcut	Uncut	Clearcut
CHLORO	30.9 (2.9)	29.7 (7.0)	25.0 (8.8)	19.7 (3.5)
METH + WATER	103.9 (16.6)	102.5 (4.4)	118.8 (5.5)	115.1 (5.0)
METH	4.4 (5.4)	18.2 (16.4)	7.2 (6.6)	7.6 (6.7)
WATER	99.5 (21.1)	84.2 (12.4)	111.6 (9.3)	107.5 (8.5)
ACID	448.5 (44.5)	447.8 (4.0)	493.8 (17.7)	522.6 (83.2)
AIR	416.7 (51.6)	420.0 (6.0)	362.4 (23.2)	342.6 (90.0)

Note: Each value is the mean of three replicates with standard deviation in parentheses. Differences between uncut and clearcut forests within stand types or between stand types within uncut forests and clearcuts were not significant.

Table 3-3. Concentration (mg g^{-1}) of C, C:N ratio and ^{13}C isotopic composition of unfractionated FH-layer forest floors and of selected proximate fractions of FH-layer forest floors from uncut and clearcut SPRUCE and ASPEN stands.

	SPRUCE		ASPEN	
	Uncut	Clearcut	Uncut	Clearcut
Unfractionated				
C	468.1 (9.0)	467.5 (12.2)	428.1 (17.7)	442.3 (28.3)
C:N	26.0 (3.1)	24.0 (0.5)	19.1 (0.8)	19.0 (1.3)
^{13}C	-26.7 (0.4)	-26.8 (0.3)	-27.8 (0.2)	-27.5 (0.2)
WATER				
C	360.0 (24.5)	357.7 (12.7)	356.7 (5.5)	352.0 (7.0)
C:N	19.8 (2.0)	19.1 (2.4)	15.2 (0.3)	15.2 (0.2)
^{13}C	-25.9 (0.2)	-26.1 (0.2)	-26.9 (0.3)	-26.9 (0.7)
AIR				
C	538.2 (14.8)	529.3 (12.2)	499.7 (20.6)	511.8 (44.3)
C:N	32.0 (1.2)	29.3 (1.7)	23.6 (1.0)	24.0 (1.7)
^{13}C	-28.0 (0.4)	-28.4 (0.2)	-29.7 (0.1)**a**	-29.3 (0.2)**b**

Note: Each value is the mean of three replicates, with standard deviation in parentheses. Within each row, values with different letters are significantly different at $P<0.05$, based on a one-way analysis of variance.

Table 3-4. Results of two-way analysis of variance of concentration of C, C:N ratio and ^{13}C isotopic composition of unfractionated FH-layer forest floors and of selected proximate fractions of FH-layer forest floors from uncut and clearcut SPRUCE and ASPEN stands.

	Source of variation	df	SS	*P*
Unfractionated				
C	Harvest (H)	1	1.38	0.54
	Stand Type (S)	1	31.92	**0.015**
	H x S	1	1.64	0.51
	Error	8	26.96	
C:N	Harvest (H)	1	3.17	0.33
	Stand Type (S)	1	108.04	**0.0003**
	H x S	1	2.78	0.36
	Error	8	23.90	
^{13}C	Harvest (H)	1	0.046	0.49
	Stand Type (S)	1	2.39	**0.0007**
	H x S	1	0.068	0.40
	Error	8	0.69	
WATER				
C	Harvest (H)	1	0.37	0.69
	Stand Type (S)	1	0.61	0.61
	H x S	1	0.043	0.89
	Error	8	16.83	
C:N	Harvest (H)	1	0.29	0.74
	Stand Type (S)	1	54.97	**0.0015**
	H x S	1	0.33	0.73
	Error	8	19.66	
^{13}C	Harvest (H)	1	0.082	0.49
	Stand Type (S)	1	2.55	**0.0037**
	H x S	1	0.033	0.66
	Error	8	1.25	
AIR				
C	Harvest (H)	1	0.075	0.92
	Stand Type (S)	1	23.42	0.10
	H x S	1	3.31	0.51
	Error	8	55.12	
C:N	Harvest (H)	1	4.09	0.20
	Stand Type (S)	1	138.71	**<0.0001**
	H x S	1	7.27	0.097
	Error	8	16.50	
^{13}C	Harvest (H)	1	0.00090	0.91
	Stand Type (S)	1	5.10	**<0.0001**
	H x S	1	0.42	**0.023**
	Error	8	0.43	

Note: Bold-faced type indicates a significant effect at $P<0.05$. df = degrees of freedom

Table 3-5. Distribution of C (% of total peak area) in ALK, O-ALK, AROM and CARB regions of CPMAS ^{13}C NMR spectra obtained from the FH-layer forest floors of uncut and clearcut SPRUCE and ASPEN stands.

	SPRUCE		ASPEN	
	Uncut	Clearcut	Uncut	Clearcut
		2002		
ALK	21.4 (1.8)	22.7 (2.1)	22.8 (1.6)	22.5 (1.6)
O-ALK	52.9 (2.1)	54.6 (2.8)	52.1 (2.0)	50.6 (2.5)
AROM	17.4 (0.2)	14.9 (2.8)	14.5 (1.5)	16.2 (1.8)
CARB	8.3 (0.4)	7.7 (1.5)	10.6 (0.4)	10.8 (0.06)
ALK:O-ALK	0.41 (0.05)	0.42 (0.03)	0.44 (0.05)	0.45 (0.05)
AROM:O-ALK	0.33 (0.02)	0.27 (0.07)	0.28 (0.04)	0.32 (0.05)
		2003		
ALK	25.3 (2.1)	24.1 (0.1)	25.8 (2.0)	25.7 (1.4)
O-ALK	58.6 (2.0)	58.4 (2.4)	56.8 (1.0)	54.6 (1.4)
AROM	11.9 (0.4)	12.7 (1.2)	11.5 (0.6)	13.1 (1.0)
CARB	4.1 (0.2)	4.7 (1.2)	6.0 (0.5)	6.6 (0.7)
ALK:O-ALK	0.43 (0.05)	0.41 (0.02)	0.45 (0.04)	0.47 (0.04)
AROM:O-ALK	0.20 (0.01)	0.22 (0.03)	0.20 (0.01)**a**	0.24 (0.02)**b**

Note: Each value is the mean of three replicates, with standard deviation in parentheses. When significant interactions were detected between stand type and harvesting treatments (Table 3-6), one-way ANOVA was used to examine harvesting effects within stand types. Within each row, values followed by different letters are significantly different at $P<0.05$, based on a one-way analysis of variance.

Table 3-6. Results of two-way analysis of variance (with year as a split effect) of the distribution of C (% of total peak area) in ALK, O-ALK, AROM and CARB regions of CPMAS ^{13}C NMR spectra obtained from FH-layer forest floors from uncut and clearcut SPRUCE and ASPEN stands.

	Source of variation	df	SS	*P*
ALK	Stand type (S)	1	3.77	0.28
	Harvest (H)	1	0.013	0.95
	S x H	1	0.18	0.81
	Error 1	8	22.13	0.54
	Year (Y)	1	49.30	**0.0035**
	Y x S	1	0.24	0.78
	Y x H	1	2.09	0.43
	Y x S x H	1	3.03	0.34
	Error 2	8	23.72	
O-ALK	Stand type (S)	1	42.21	**0.0048**
	Harvest (H)	1	1.65	0.47
	S x H	1	10.10	0.96
	Error 1	8	22.73	0.84
	Year (Y)	1	123.87	**0.0018**
	Y x S	1	0.19	0.86
	Y x H	1	2.30	0.55
	Y x S x H	1	0.57	0.76
	Error 2	8	46.97	
AROM	Stand type (S)	1	1.03	0.40
	Harvest (H)	1	0.69	0.49
	S x H	1	9.51	**0.028**
	Error 1	8	10.57	0.83
	Year (Y)	1	71.11	**0.0009**
	Y x S	1	0.85	0.59
	Y x H	1	3.87	0.26
	Y x S x H	1	4.24	0.24
	Error 2	8	21.52	
CARB	Stand type (S)	1	31.00	**<0.0001**
	Harvest (H)	1	0.33	0.29
	S x H	1	0.26	0.34
	Error 1	8	2.03	0.96
	Year (Y)	1	94.40	**<0.0001**
	Y x S	1	0.97	0.34
	Y x H	1	0.99	0.33
	Y x S x H	1	0.19	0.67
	Error 2	8	7.50	

Table 3-6. (continued)

ALK:O-ALK	Stand type (S)	1	0.0076	0.070
	Harvest (H)	1	0.000083	0.83
	S x H	1	0.0036	0.66
	Error 1	8	0.0014	0.54
	Year (Y)	1	0.0015	0.40
	Y x S	1	0.000081	0.84
	Y x H	1	0.00018	0.77
	Y x S x H	1	0.00062	0.58
	Error 2	8	0.015	
AROM:O-ALK	Stand type (S)	1	0.00011	0.72
	Harvest (H)	1	0.00048	0.45
	S x H	1	0.0054	**0.030**
	Error 1	8	0.0062	0.83
	Year (Y)	1	0.044	**0.0007**
	Y x S	1	0.00019	0.74
	Y x H	1	0.0016	0.35
	Y x S x H	1	0.0020	0.29
	Error 2	8	0.013	

Note: Bold-faced type indicates a significant effect at $P<0.05$. df = degrees of freedom

Table 3-7. Selected correlation coefficients (Pearson's *r*) describing the relationships between the results of proximate fractionation (mg g^{-1}), ^{13}C isotopic determination and CPMAS ^{13}C NMR spectroscopy (% of total peak area) of FH-layer forest floors from a SPRUCE clearcut and uncut stand and an ASPEN clearcut and uncut stand.

	CHLORO		METH + WATER		ACID		AIR		^{13}C-AIR	
	r	*P*	r	*P*	r	*P*	r	*P*	r	*P*
ALK	-0.28	0.18	-0.37	0.084	0.00064	0.99	0.37	0.072	**-0.42**	0.040
O-ALK	-0.040	0.85	0.22	0.32	0.35	0.095	**-0.53**	0.0082	0.22	0.31
AROM	0.30	0.15	0.39	0.064	-0.038	0.86	-0.21	0.32	**0.62**	0.0011
CARB	0.075	0.72	-0.29	0.17	**-0.45**	0.027	**0.45**	0.026	**-0.52**	0.010
^{13}C-AIR	-0.57	0.79	**0.51**	0.014	0.22	0.30	-0.37	0.075		

Note: Bold-faced type indicates a significant relationship (n=24) at $P<0.05$. The adjusted *P*-value using a Bonferroni correction is 0.0021.

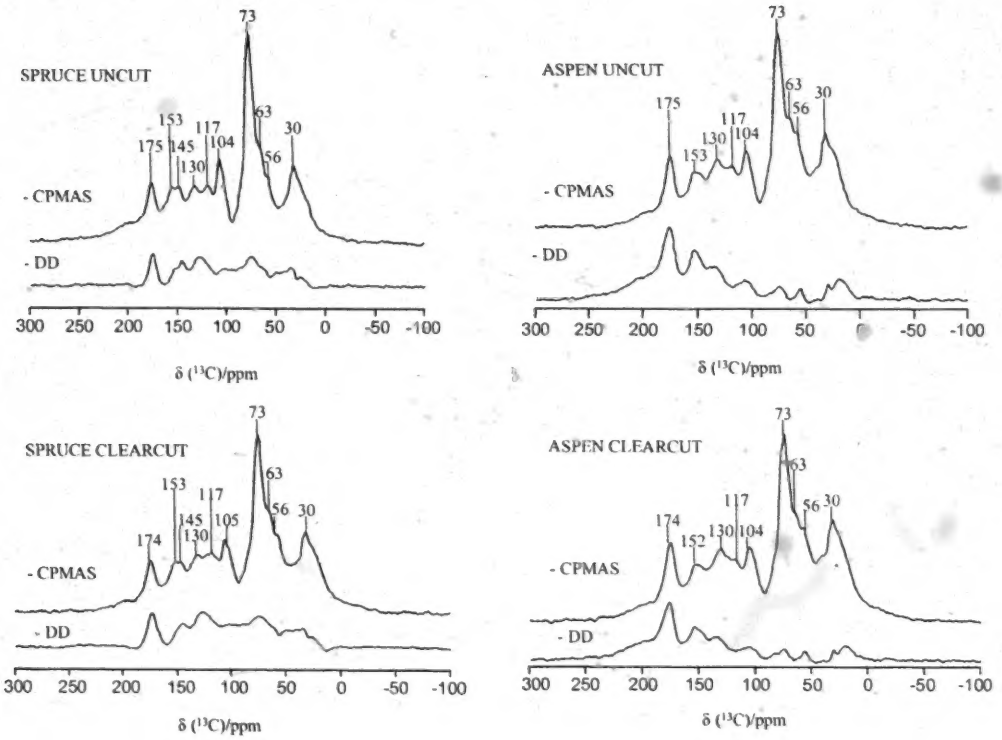

Figure 3-1. Representative cross-polarization magic-angle spinning (CPMAS) and dipolar-dephased (DD) ^{13}C NMR spectra of FH-layer forest floors from uncut and clearcut SPRUCE stands and from uncut and clearcut ASPEN stands.

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Chapter 4 Forest floor microbial communities in relation to stand composition and timber harvesting in northern Alberta

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Introduction

With the recent proliferation in the availability of techniques for examining microbial community structure in soils, several recent studies have determined that not only can different tree species influence the chemical properties of forest soils (e.g., Bradley et al., 1997; Priha et al,. 1999; Thomas and Prescott, 2000) but also the composition of forest soil microbial communities. A pot study examining the influence on soil properties of three species of tree seedlings revealed that after only 3 months the composition of microbial phospholipid fatty acids (PLFAs) and the community-level physiological profiles (CLPP; determined using Biolog® plates) of organic soil planted to silver birch (*Betula pendula* Roth) were considerably different from those of organic soil planted to Scots pine (*Pinus sylvestris* L.) or Norway spruce (*Picea abies* (L.) Karst) (Priha et al., 1999). In two 55 to 60 year-old common garden experiments in Finland, soils planted to Scots pine, Norway spruce or silver birch supported microbial communities with distinct PLFA compositions but no difference in CLPPs (Priha et al., 2001). Forest floors from 40 year-old plantations of western redcedar (*Thuja plicata* Donn ex. Don.), western hemlock (*Tsuga heterophylla* (Raf.) Sarg.), Douglas-fir (*Pseudotsuga menziesii* (Mirb.) Franco) and Sitka spruce (*Picea sitchensis* (Bong.) Carr) at three sites on Vancouver Island, Canada, also supported different microbial communities, assessed using PLFAs and CLPPs, although the effects of site and forest floor layer interacted strongly with species effects (Grayston and Prescott, 2005). Finally, the results of PLFA and ribosomal intergenic spacer analysis (RISA) indicated that forest floors from adjacent stands of western redcedar-western hemlock and western hemlock-amabilis fir (*Abies amabilis* (Dougl.) Forbes) on Vancouver Island were characterized by different fungal community structures and proportions of Gram + bacteria (Leckie et al., 2004).

The composition of the soil microbial community in different forest types may influence post-disturbance rates of nutrient cycling and, ultimately, regeneration success because soils with distinct patterns in microbial community structure also frequently exhibit differences in nutrient dynamics (Priha et al., 1999; Priha et al., 2001; Leckie et al., 2004; Grayston and Prescott, 2005). Therefore, it is critical to understand how timber harvesting influences the soil microbial community in stands of varying tree species composition. Furthermore, the growing interest in forest management that more closely emulates the effects of natural disturbance has created a need for research that examines the influence on soil microbial communities of silvicultural systems that retain some portion of the original stand (Spence, 2001; Bergeron et al., 2004). The few studies comparing the effects of different silvicultural systems on the soil microbial community have reported conflicting results in forests of different tree species composition (Siira-Pietikäinen et al., 2001a; Lindo and Visser, 2003; Brais et al., 2004). Thus, further work is required to compare the effects of clearcutting and partial harvesting on the forest soil microbial communities of different stand types.

The Ecosystem Management Emulating Natural Disturbance (EMEND) experiment, in northwestern Alberta, is a long-term research study covering over 1000 ha of boreal mixedwood forest that has been divided into stands dominated by deciduous species, particularly trembling aspen (*Populus tremuloides* Michx.), stands dominated by coniferous species, particularly white spruce (*Picea glauca* (Moench) Voss), and stands with approximately equal proportions of coniferous and deciduous trees. This controlled and replicated experiment was established, in part, to examine differences among stand types in the response of ecosystem-level processes to varying intensities of timber harvesting. Harvesting treatments include uncut controls, partial harvests in which 50% of the original stand was retained (50% retention), partial harvests in which 20% of the original stand was retained (20% retention), and clearcuts. The aims of the present study were: i. to determine if the structure and biomass of the forest floor microbial community differed among stand types, ii. to determine whether partial harvesting mitigated post-harvest changes in microbial community structure and biomass, compared with clearcutting, and iii. to determine whether post-harvest changes in the properties of the forest floor microbial community varied among stand types.

Materials and Methods

Study site

The EMEND site (56° 46' 13" N, 118° 22' 28" W) is located on the boreal mixedwood plain in the Clear Hills Upland Ecoregion within the Boreal Plains EcoZone (Wiken, 1986; EcoRegions Working Group, 1989). The area is characterized by cold winters (mean temperature –14.0°C), warm summers (mean temperature 11.6°C), and an average 433 mm of precipitation, 2/3 of which usually falls during the summer (Environment Canada, 2005). The site has a rolling topography ranging in elevation from 677 to 880 m asl. Soils are usually Brunisols, Orthic Gray Luvisols or Dark Gray Luvisols that have developed on fine-textured glacio-lacustrine or glacial till parent materials (Kishchuk, 2004).

Undisturbed stands at EMEND range in age from 70 to 130 years of age (Spence and Volney, 1999). Although this is not always the case, trembling aspen generally dominates younger stands of the boreal mixedwood forest; white spruce becomes dominant as these stands age (Rowe, 1956). Undisturbed white spruce-dominated (SPRUCE) stands consist of > 70% white spruce, with some trembling aspen, balsam poplar (*Populus balsamifera*), paper birch (*Betula papyrifera*), balsam fir (*Abies balsamea*), or lodgepole pine (*Pinus contorta*). The understory of SPRUCE stands includes *Rosa acicularis*, *Sheperdia canadensis* and a dense ground cover of moss, especially *Hylocomium splendens*, *Pleurozium schreberi* and *Ptilium crista-castrensis*. Forest floors in SPRUCE stands are typically Humimors (Green et al., 1993). Undisturbed trembling aspen-dominated (ASPEN) stands consist of > 70% trembling aspen, with some of the tree species listed above for SPRUCE stands. The understory of ASPEN stands includes *Rosa acicularis*, *Viburnum edule*, and *Alnus* spp, with an herb layer of *Calamagrostis canadensis*, *Chamerion angustifolium* and *Cornus canadensis*. Forest floors in ASPEN stands are typically Mormoders (Green et al., 1993). Undisturbed MIXED stands consist of approximately equivalent proportions of coniferous and deciduous tree species, with small patches dominated by either coniferous or deciduous trees. The composition of the understory vegetation in MIXED stands tends to be more similar to that in SPRUCE

stands (Fenniak, unpublished thesis, 2001). Selected descriptive data for the FH-layer forest floors of SPRUCE, ASPEN and MIXED stands are provided in Table 4-1.

Harvesting of the clearcuts, and partial retention cuts, which are each approximately 10 ha in size, was completed in the winter of 1998-1999, when the ground was frozen, in order to minimize disturbance of the soil during timber removal. In the clearcuts, whole trees were harvested using a feller-buncher and skidded directly to the landing, where cut stems were de-limbed. In the partial cuts, whole trees were harvested in a two-pass system using a feller-buncher. In the first pass, all of the trees were removed from 5 m-wide machine corridors that were oriented in a north-south direction (perpendicular to the prevailing wind) and spaced 20 m apart. In the second pass, one out of every three (50% retention) or three out of every four (20% retention) stems were removed by reaching into the remaining forest from the machine corridors using a feller-buncher. Harvested trees were skidded to the landing along the machine corridors. Following clearcutting and partial cutting, debris from the de-limbing process was piled on the landing and burned (Sidders and Spence, 2001). Site preparation, e.g., scarification or burning, was not applied in any of the sampling areas and vegetation has been allowed to regenerate naturally.

Sample collection

In June 2003, samples of forest floor were collected from three 10-ha replicates each of uncut (undisturbed), 50% retention, 20% retention and clearcut SPRUCE, ASPEN and MIXED stands (a total of 36 experimental units) for assessment of PLFA profiles. In June 2004, samples of forest floor were collected from three 10-ha replicates each of uncut, 50% retention and clearcut SPRUCE, ASPEN and MIXED stands (total of 27 experimental units) for assessment of substrate-induced respiration (SIR) profiles. Forest floor samples were not collected from the 20% retention treatments for assessment of SIR in order to ensure that analyses were completed in a timely manner.

Six sampling sites were randomly assigned within each experimental unit. Some prospective sampling sites were rejected in order to avoid visibly disturbed forest floors and rotten wood. At each sampling site, the FH-layer forest floor was excavated to the depth of the mineral soil surface. The F- and H-layers of the forest floor were collected together because the F-layer was very thin in ASPEN stands and, therefore, difficult to

separate from the H-layer. In SPRUCE stands, the bulk of the forest floor was dominated by decomposing moss, which made it difficult to distinguish the boundary between the F- and the H-layers.

PLFA analyses

FH-layer material collected for PLFA analysis was placed in a sterile plastic bag and kept on ice until the end of the day, when it was sieved (4 mm) to remove roots and twigs, and frozen on dry ice. Upon transport to the laboratory, frozen forest floor samples were stored at –86 °C. Because of resource limitations, three of the six samples of sieved, frozen FH-layer forest floor that had been collected from each experimental unit were randomly selected for PLFA analysis and freeze-dried. Polar lipids were extracted from 300 mg aliquots of freeze-dried FH-layer forest floor material using a modified Bligh and Dyer extraction (Bligh and Dyer, 1959; Frostegård et al., 1991; White and Ringelberg, 1998). Polar lipid extracts were purified on pre-packed silicic acid columns (Agilent Technologies, Wilmington, DE) and subjected to mild alkaline methanolysis to form fatty acid methyl esters (FAMEs). FAMEs were separated and quantified using an Agilent 6890 Series capillary gas chromatograph (Agilent Technologies, Wilmington, DE) equipped with a 25 m Ultra 2 (5%-phenyl)-methylpolysiloxane column. Hydrogen was used as the carrier gas. Peaks were identified using bacterial fatty acid standards and MIDI peak identification software (MIDI, Inc., Newark, DE).

Fatty acids were designated X:YωZ, where X represents the number of carbon atoms, Y represents the number of double bonds, and Z indicates the position of the first double bond from the aliphatic (ω) end of the molecule. Prefixes 'i' and 'a' indicate branching at the second or third carbon atom, respectively, from the ω end. The prefix 'cy' indicates the presence of a cyclopropyl group somewhere along the carbon chain, while '10Me' indicates a methyl group at the tenth carbon atom from the carboxyl end of the molecule. Cis or trans configurations are indicated by the suffixes 'c' or 't', respectively. Only fatty acids of less than 20 carbons in chain length were included in the calculation of total microbial PLFAs.

Total phospholipid fatty acids were compared among stand types and harvesting treatments as nmol g^{-1} forest floor, while PLFAs used as biomarkers (Table 4-2; Frostegård and Bååth, 1996; Hassett and Zak, 2005) were compared on a mole % basis in

order to standardize for differences in the total amount of forest floor PLFAs among stand types. Indices of community structure, including PLFA richness, diversity and evenness were also calculated. PLFA richness indicates the total number of PLFAs detected that were less than 20 carbons in chain length. PLFA diversity was calculated using the Shannon index, where $H' = -\Sigma(p_i \times \log(p_i))$ and p_i is the mole fraction of individual PLFAs. PLFA evenness, a measure of the variability in the abundance of different PLFAs within a sample, was calculated as $E = H'/\ln(\text{PLFA richness})$ (Schutter and Dick, 2001).

SIR analyses

At each sampling site, FH-layer material collected for SIR analysis was placed in a sterile plastic bag and kept on ice. Upon transport to the laboratory, FH-layer materials collected from the six sampling sites within each experimental unit were sieved (4 mm) to remove large roots and twigs, and composited into one sample. All 27 composited samples were brought to equivalent moisture contents (3.9 mL water g^{-1} forest floor) and stored at approximately 5 °C for three weeks.

SIR profiles of the FH-layer forest floor microbial community were obtained using a modified version of the technique developed by Degens and Harris (1997). This technique was designed to eliminate some of the problems associated with the CLPP technique, and appears to be more sensitive to changes in land management (Graham and Haynes, 2005). Twenty-one carbon compounds were selected as test substrates based on their ability to discriminate between treatments in previous studies (Degens and Harris, 1997) and their availability in the laboratory (Table 4-3). Two weeks prior to analysis, samples were removed from the refrigerator and incubated in the dark at room temperature (Degens et al., 2001). The day before analysis, duplicate 1 g (dry weight equivalent) aliquots of forest floor were dispensed into 140 mL glass vials and sealed with Parafilm. Solutions containing the test substrates were brought to the desired concentration ((Degens and Vojvodić-Vuković, 1999; Table 4-3) after adjusting solution pH to between 6.5 and 7.0 using concentrated NaOH or HCl (Degens, 1998) SIR profiles were determined by measuring the carbon dioxide respired by the forest floor material in each vial after the addition of 1 mL of distilled deionised water (as a control) or 1 mL of a solution containing a test substrate, which raised the moisture of the forest floor in each

vial to 4.9 mL g^{-1}, or approximately 60% water-holding capacity. Immediately after addition of the solution, each vial was sealed with a rubber septum, vortexed, and incubated in a water bath at 25°C for one hour. Samples were vortexed a second time immediately prior to analysis. Head-space carbon dioxide concentrations were determined using a Hewlett Packard 5890 Series II gas chromatograph equipped with a 1m Poropak Q column, a HP3396 Series II integrator and helium as the carrier gas. A one-hour incubation was employed in this study because data from a pre-trial indicated that large increases or decreases in microbial biomass occurred when these forest floors were incubated for more than an hour (data not shown).

Catabolic evenness, as an index of community structure, was calculated from the SIR data as $E = 1/\Sigma p_i^2$ (Magurran, 1988) where $p_i = r_i/\Sigma r_i$, i.e., the SIR of each test substrate (r_i) as a proportion of the total SIR summed over all test substrates (Σr_i; Degens et al., 2001).

Statistical analysis

Two-way (harvesting treatment x stand type) factorial analyses of variance (ANOVA) for a completely randomized design were used to examine patterns in total PLFA (nmol g^{-1}), biomarker PLFAs (mole %), indices of community structure and basal respiration rates (μg CO_2-C g^{-1} forest floor h^{-1}). Differences were considered statistically significant if $P<0.05$. None of these data required transformation to meet the assumptions of the analyses. ANOVAs were performed using SAS (version 8.01, SAS Institute Inc. 1999-2000, Cary, NC).

Patterns of forest floor PLFAs (mole %, after arcsine square-root transformation) and SIR in response to the addition of the 21 individual test substrates (after standardization; Degens et al., 2001) among stand types and harvesting treatments were examined with the non-metric multidimensional scaling (NMS) ordination technique (Kruskal, 1964; Mather, 1976) using PC-ORD software (version 4, MjM Software Design, Gleneden Beach, OR). NMS is an unconstrained ordination method that iteratively searches for the best way to represent the data in a reduced number of dimensions so that the distances in the ordination diagram reflect the similarities or dissimilarities in community structure of the original samples. The Sorensen (Bray-Curtis) distance measure was used in the analyses. NMS is considered better than many other ordination techniques because it

does not assume a linear relationship among variables (Clarke, 1993). The multiple response permutations procedure (MRPP) was used to compare the distances in the ordination space between points corresponding to different stand types or harvesting treatments in order to determine whether these groups were statistically different. Bonferroni corrections were used to determine a family error rate (Legendre and Legendre, 1998). Indicator species analysis (Dufrêne and Legendre, 1997) was used to select individual PLFAs whose presence may be used to indicate specific stand types. As its name implies, this technique is generally used for species data. However, given that the method integrates data on both the concentration and relative frequency of a 'species' within a group (McCune and Grace, 2002), the method is also appropriate for PLFA data.

Results

PLFA analyses

In all, 74 PLFAs were included in the analysis of total PLFAs, which ranged from an average of 816 nmol g^{-1} in forest floor from MIXED stands subjected to 20% retention, to 1626 nmol g^{-1}, in forest floor from uncut ASPEN stands (Figure 4-1a). PLFA concentrations in forest floors from ASPEN stands were significantly higher than in forest floors from MIXED or SPRUCE stands (Table 4-4; $P<0.0001$). There were no effects of harvesting on the total amount of PLFAs from forest floors either across or within stand types.

The mole % of fungal PLFAs ranged from approximately 13% to 18%, while bacterial PLFAs ranged from approximately 33% to 37% (Table 4-5). There were no differences among stand types or harvesting treatments in the mole % of fungal or bacterial PLFAs, the mole % of Gram + or Gram – bacteria or in the ratio of fungal/bacterial PLFAs (Table 4-4). However, there was a higher proportion of PLFAs associated with actinomycetes in forest floors from ASPEN stands than in forest floors from SPRUCE stands (Figure 4-1b; $P=0.0002$). Furthermore, the mole % of the PLFA 16:1ω5, frequently associated with arbuscular mycorrhizal fungi and Gram – bacteria, was significantly greater in forest floors from ASPEN stands than from MIXED or SPRUCE stands (Figure 4-1c; $P=0.036$), and in forest floors from clearcut stands than from uncut stands ($P=0.012$).

The indices of community structure, calculated using the PLFA data, also showed strong differences among stand types but not among harvesting treatments (Table 4-6). The diversity of PLFAs, which ranged from approximately 1.70 to 1.80, was significantly greater (P=0.0014) in forest floors from SPRUCE and MIXED stands than in forest floors from ASPEN stands (Table 4-7). Although there were no differences among stand types or harvesting treatments in PLFA richness, the effect of stand type on PLFA evenness, which integrates both diversity and richness, was also statistically significant (P=0.036). However, the Bonferroni test failed to distinguish differences in PLFA evenness among stand types.

In all, 61 PLFAs were used in the NMS analysis (Figure 4-2). According to the MRPP analyses, the pattern of PLFAs in ASPEN forest floors differed significantly from MIXED or SPRUCE forest floors in the NMS ordination (A=0.13, P=0.000056 and A=0.22, P=0.0000041, respectively), but MIXED and SPRUCE forest floors were not different from each other (A=0.016. P=0.10). The family error rate, obtained using the Bonferroni correction, was P<0.0056. ASPEN forest floors separated, for the most part, from SPRUCE and MIXED forest floors along both axes 1 and 2 (Figure 4-2). There was no clear effect of harvesting on PLFA profiles in these forest floors, even when stand types were analyzed separately (data not shown).

The correlation between forest floor PLFA profiles and stand or forest floor descriptive variables is also shown in Figure 4-2. The PLFAs associated with ASPEN forest floors were most strongly correlated with forest floor pH (axis1, r = 0.71; axis 2, r = 0.78), forest floor N concentration (axis 1, r = 0.77, axis 2, r = 0.75), and the height of regenerating aspen and balsam poplar trees (axis 1, r = 0.47, axis 2, r = 0.57). The PLFAs associated with SPRUCE and MIXED forest floors were more strongly associated with the percent cover of low shrubs (axis 1, r = -0.71; axis 2, r = -0.55) and moss (axis 1, r = -0.59; axis 2, r = -0.72) and the basal area of conifers (axis 1, r = -0.48; axis 2, r = -0.74). The basal area of deciduous trees and the percent cover of tall shrubs, alder, grass and herbs showed little relationship with the PLFA profiles of these forest floors (r < 0.40).

Indicator analysis of these data detected six PLFAs indicative of ASPEN or SPRUCE stands (levels of these PLFAs tended to be intermediate in forest floors from

MIXED stands) but none of these are considered biomarkers for a specific group of microbes (Table 4-8). Indicator analysis of the data did not detect any PLFAs that were useful as indicators of harvesting effects.

SIR analyses

Mean rates of basal respiration ranged from 49.3 μg CO_2-C g^{-1} forest floor h^{-1}, in forest floors from ASPEN clearcuts, to 78.2 μg CO_2-C g^{-1} forest floor h^{-1} in forest floors from MIXED uncut stands (Figure 4-1d). There was no significant trend in basal respiration rates among harvesting treatments within or across stands. However, basal respiration rates overall were highest in forest floors from MIXED stands and lowest in forest floors from ASPEN stands (P=0.029; Table 4-4), where basal respiration rates were most reduced by harvesting (although this trend was not statistically significant). Catabolic evenness ranged from 18.5 in forest floors from uncut MIXED stands to 20.0 in forest floors from clearcut and 50% retention ASPEN stands (Table 4-6). Although catabolic evenness didn't vary strongly across harvesting treatments, it was significantly higher (Table 4-7; P=0.0043) in forest floors from ASPEN stands than in forest floors from MIXED or SPRUCE stands.

The pattern of SIR (Figure 4-3) in ASPEN forest floors differed significantly from that in MIXED and SPRUCE forest floors (A=0.13, P=0.0011; A=0.13, P=0.00027, respectively) but MIXED and SPRUCE forest floors did not differ from each other (A=0.021, P=0.11). The family error rate, obtained using the Bonferroni correction, was P<0.0083. ASPEN forest floors separated, for the most part, from SPRUCE and MIXED forest floors along both axes 1 and 2 (Figure 4-3). There was no clear effect of harvesting on SIR profiles in these forest floors, even when stand types were analyzed separately (data not shown).

The correlation between forest floor SIR profile and stand or forest floor descriptive variables is also shown in Figure 4-3. The SIR response of forest floors from ASPEN stands was most strongly correlated with forest floor pH (axis1, r = -0.54; axis 2, r = -0.82), forest floor N concentration (axis 1, r = -0.27, axis 2, r = -0.70), the height of regenerating aspen and balsam poplar trees (axis 1, r = -0.20, axis 2, r-0.62) and the percent cover of tall shrubs (axis 1, r = 0.07, axis 2, r = -0.49). The SIR response of forest floors from SPRUCE and MIXED stands was most strongly associated with the percent

cover of low shrubs (axis 1, $r = 0.25$; axis 2, $r = -0.63$) and moss (axis 1, $r = 0.51$; axis 2, $r = 0.59$) and the basal area of conifers (axis 1, $r = 0.59$; axis 2, $r = 0.60$). The basal area of deciduous trees and the percent cover of alder, grass and herbs showed little relationship with the SIR response of these forest floors ($r < 0.40$).

Discussion

Differences in microbial biomass and microbial community structure among stand types

ASPEN forest floors supported a greater microbial biomass (concentration of total PLFAs) and a different microbial community structure (as indicated by both the PLFA and SIR analyses) than MIXED or SPRUCE forest floors. Previous studies that have also detected higher concentrations of microbial biomass in ASPEN forest floors have largely attributed this pattern to differences in initial litter quality (Flanagan and Van Cleve, 1983; Lindo and Visser, 2003). However, the decay rates of white spruce needles and trembling aspen leaves appear to converge within four or five years (Prescott et al., 2000; Prescott et al., 2004). Thus, initial litter quality does not sufficiently explain differences in the patterns of microbial biomass in the FH-layer forest floors of these stands. Instead, it is more likely that a combination of factors interact to produce forest floor microbial communities with unique characteristics that are a function of differences in initial litter chemistries, quantities and qualities of fine root inputs (Ruess et al., 1996; Finér et al., 1997; Lindo and Visser, 2003), populations of soil fauna (Prescott et al., 2000; Lindo and Visser, 2004), and soil moisture and temperature regimes (Fox and Van Cleve, 1983; Fenniak, unpublished thesis, 2001; Lindo and Visser, 2003). The observation that ASPEN forest floors usually (Flanagan and Van Cleve, 1983; Lindo and Visser, 2003), though not always (Côté et al., 2000), exhibit greater rates of C, N and P mineralization under standardized conditions supports the idea that, regardless of the combination of mechanisms involved, ASPEN stands tend to produce forest floors that support a greater microbial biomass than SPRUCE stands.

The similarities in microbial biomass, indices of community structure and microbial community composition between forest floors from SPRUCE and MIXED stands were somewhat surprising. In MIXED stands, these variables were expected to be

either intermediate between the other two stand types or, given that soils from MIXED stands are widely considered more productive than soils from pure stands (Man and Lieffers, 1999), larger and more diverse than in either ASPEN or SPRUCE stands. However, the similarity in the community structure and microbial community composition of MIXED and SPRUCE forest floors is mirrored by the findings of Fenniak (unpublished thesis, 2001) and Work et al. (2004), who observed that the compositions of the understory vascular plant community and the epigaeic arthropod community in MIXED and SPRUCE stands at EMEND were relatively similar to each other but very different from those in ASPEN stands. Catabolic evenness, an indicator of the flexibility of the forest floor microbial community to degrade different C sources, is expected to increase with the variety of organic materials available for degradation (Degens et al., 2000; Schutter and Dick, 2001; Carney and Matson, 2005). Therefore, the catabolic evenness of the microbial community in these forest floors may actually reflect the diversity of the understory vegetation (Priha et al., 2001), which is highest in ASPEN stands at EMEND (Fenniak, unpublished thesis, 2001). Nilsson and Wardle (2005) recently showed that the composition of the understory vegetation exerts a strong influence on decomposition and nutrient cycling patterns in Swedish boreal forest soils.

Of the six PLFAs with high indicator values, five were found exclusively in SPRUCE or MIXED forest floors, which suggests that white spruce (or the understory associated with white spruce) may have a strong influence over the PLFA profile of these forest floors. Indeed, the results of previous studies suggest that coniferous trees exert a stronger influence over the microbial community structure of forest soils than deciduous trees (Saetre and Bååth, 2000; White et al., 2005). This may explain why the MIXED and SPRUCE stands tended to group together in the ordinations of both the PLFA and SIR data (Figure 4-1 and Figure 4-2). Such an idea is also supported by the fact that the basal area of coniferous trees (which were predominantly white spruce) consistently showed strong correlations with axes 1 and 2 in both ordinations while the basal area of deciduous trees did not. Thus, the influence of both the overstory and understory vegetation on the forest floor microbial community in these stands requires further investigation.

Changes in microbial biomass and microbial community composition induced by timber harvesting

In general, no effects of timber harvesting on total microbial biomass, microbial community composition, indices of community structure or basal respiration rates were detected either across or within stands. There are numerous possible explanations for the lack of differences in these variables among harvesting treatments. Samples were collected for PLFA and SIR analysis on only one date each and the strength of harvesting effects could have changed through the season. However, the results of previous studies that have compared soil microbial communities on multiple sample dates suggest that although microbial community composition can be quite variable through the season, differences among treatments tend to remain consistent (Grayston et al., 2001; Myers et al., 2001). PLFA and SIR analyses may also not have been sufficiently sensitive to detect shifts in microbial community composition induced by the harvesting treatments. Indeed, large changes in catabolic evenness tend to arise only when management practices induce significant reductions in soil organic C concentrations (Degens et al., 2000; Graham and Haynes, 2005), which did not occur in this study (Table 4-1). However, changes in the amount and composition of microbial PLFAs have been reported in the first few years following harvesting in previous studies (Bååth et al., 1995; Siira-Pietikäinen et al., 2001a). Therefore, it does not seem likely that a lack of sensitivity in both the PLFA and SIR analyses is the reason that harvesting effects were not detected in this study.

Significant reductions in forest floor microbial biomass had been observed in clearcut ASPEN and SPRUCE stands at the EMEND site 2.5 years after harvesting (Lindo and Visser, 2003). However, these changes had disappeared the following year (Lucie Jerabkova, personal communication, University of British Columbia) and remained undetectable 4.5 years after harvesting, when samples were collected for PLFA analysis in the present study. Therefore, it seems likely that the forest floor microbial community had responded immediately after harvesting but had returned to pre-harvest levels by the time sampling was performed 4.5 and 5.5 years post-harvest (for PLFA and SIR analysis, respectively). The composition of the forest soil microbial community can rebound to pre-harvest levels within the first few years after harvesting (Siira-Pietikäinen et al., 2001a). However, the rapid return to pre-harvest levels of microbial biomass

remains a remarkable recovery rate given that reduced microbial biomass and altered PLFA biomarker concentrations have been reported in soils from other boreal forests 5-10 years post-harvest (Bååth, 1980; Pennanen et al., 1999; Hassett and Zak, 2005). It is possible that the efforts to reduce soil disturbance and facilitate natural revegetation at EMEND (see Methods section) preserved the resilience of the forest floor microbial communities to disturbance.

Unlike any of the other PLFA biomarkers, the relative abundance of 16:1ω5 was significantly greater in forest floor from clearcuts and 50% retention treatments than in forest floors from uncut controls. This PLFA was included in the list of biomarkers for Gram – bacteria (Table 4-2) and is also frequently used as an indicator of the presence of arbuscular mycorrhizal fungi (Olsson et al., 1999). Concentrations of 16:1ω5 have been reported to increase in forest soils after clearcutting (Bååth et al., 1995; Pennanen et al., 1999) and root-severing treatments (Siira-Pietikäinen et al., 2001b). Such changes are believed to result from the inter-related effects of elevated pH, invasion by grasses, increased abundance of arbuscular mycorrhizal fungi and Gram – bacteria, and/or enhanced availability of C and nutrients (Bååth et al., 1995; Pennanen et al., 1999; Priha et al., 1999; Saetre and Bååth, 2000; Siira-Pietikäinen et al., 2001b; Merilä et al., 2002). It is not possible to determine conclusively which factors caused the increased abundance of this PLFA in the present study. However, both the pH of the forest floor (Table 4-1) and the percent cover of grasses (L. Jerbakova, personal communication, University of British Columbia) increased with harvesting in all stand types.

Conclusions

The aims of the present study were: i. to determine if the structure and biomass of the forest floor microbial community differed among stand types, ii. to determine whether partial harvesting mitigated post-harvest changes in microbial community structure and biomass, compared with clearcutting, and iii. to determine whether post-harvest changes in the properties of the forest floor microbial community varied among stand types. ASPEN forest floors supported a greater microbial biomass and a very different microbial community structure than MIXED or SPRUCE forest floors. This pattern may reflect stand-type differences in the composition of the understory vegetation community. Furthermore, the presence of white spruce appears to have a strong influence on

microbial community structure. Somewhat surprisingly, the forest floor microbial community in all three stand types appeared to be unchanged by either partial or clearcut harvesting 4.5 and 5.5 years post-harvest. From the perspective of the forest floor microbial community, partial harvesting does not appear to have a benefit over clearcut harvesting in any stand type at EMEND, although this resilience to disturbance may be the result of the careful harvesting techniques used to minimize soil disturbance in the study area.

Tables and Figures

Table 4-1. Selected characteristics of FH-layer forest floors from SPRUCE, MIXED and ASPEN clearcuts (0%), and 20% retention, 50% retention and uncut control (100%) treatments.

	SPRUCE				MIXED				ASPEN			
	0%	20%	50%	100%	0%	20%	50%	100%	0%	20%	50%	100%
C (mg g^{-1})	467.5	439.0	454.0	466.8	461.6	439.3	444.6	416.2	442.3	438.7	441.2	428.1
	(7.0)	(10.9)	(8.3)	(6.3)	(3.3)	(19.5)	(7.6)	(24.9)	(16.4)	(3.5)	(11.3)	(10.2)
N (mg g^{-1})	19.4	19.3	18.7	18.1	19.9	18.5	20.1	19.8	23.3	24.7	25.4	22.5
	(0.5)	(1.7)	(2.0)	(1.0)	(1.9)	(1.2)	(2.3)	(1.8)	(0.5)	(0.9)	(0.8)	(0.1)
Thickness	0.10	0.11	0.11	0.14	0.080	0.12	0.097	0.093	0.095	0.079	0.10	0.077
(m)	(0.001)	(0.001)	(0.001)	(0.001)	(0.001)	(0.001)	(0.001)	(0.002)	(0.001)	(0.001)	(0.002)	(0.005)
ρ_b (Mg m^{-3})	0.069	0.068	0.060	0.057	0.082	0.070	0.069	0.065	0.097	0.088	0.094	0.081
	(0.010)	(0.007)	(0.008)	(0.003)	(0.012)	(0.015)	(0.009)	(0.013)	(0.006)	(0.008)	(0.006)	(0.005)
pH	5.1	5.1	5.0	4.3	5.2	5.0	5.2	4.9	6.0	5.9	6.3	5.6
	(0.3)	(0.1)	(0.01)	(0.3)	(0.3)	(0.2)	(0.3)	(0.2)	(0.2)	(0.3)	(0.1)	(0.1)

Note: Values are means (n=3) with standard error in parentheses.

Table 4-2. PLFAs used as biomarkers

Microbial Group	PLFA Marker
Fungi	18:1ω9c
	18:2ω6 + a18:0
Bacteria	i15:0
	a 15:0
	15:0
	i16:0
	i17:0
	a17:0
	17:0
	cy17:0
	18:1ω7
	cy19:0
Gram +	i15:0
	a15:0
	i16:0
	10me16:0
	a17:0
	i17:0
Gram –	16:1ω5c
	cy17:0
	cy19:0
	18:1ω5c
	18:1ω7c
Actinomycetes	10me18:0

Table 4-3. Sole carbon sources used to measure SIR profiles

Type	Concentration (M)	Compound
Amino Acid	0.015	L-arginine
	0.015	L-asparagine
	0.015	L-glutamic Acid
	0.015	L-histidine
	0.015	DL-lysine
	0.015	DL-serine
Aromatic	0.015	Urocanic Acid
Carbohydrate	0.075	D-glucose
	0.075	D-mannose
	0.075	Sucrose
Carboxylic Acid	0.05	α-ketovaleric Acid
	0.075	Uric Acid
	0.1	L-ascorbic Acid
	0.1	Citric Acid
	0.1	D-gluconic Acid
	0.1	α-ketoglutaric Acid
	0.1	DL-malic Acid
	0.1	Pantothenic Acid
	0.1	(-)-Quinic Acid
	0.1	Succinic Acid
	0.1	DL-tartaric Acid

Table 4-4. Results of analyses of variance examining the effects of stand type and harvesting treatments on total PLFAs, PLFA biomarkers and basal respiration rates in FH-layer forest floors.

Variable	Factor	df	MS	*P*
Total PLFAs	Stand Type (S)	2	671067.8	**<0.0001**
	Harvest (H)	3	49168.0	0.32
	S x H	6	38471.1	0.47
	Error	24	39650.5	
Fungi	Stand Type (S)	2	0.57	0.92
	Harvest (H)	3	2.64	0.75
	S x H	6	6.42	0.46
	Error	24	6.58	
Bacteria	Stand Type (S)	2	0.23	0.93
	Harvest (H)	3	0.32	0.96
	S x H	6	5.73	0.17
	Error	24	3.38	
Gram +	Stand Type (S)	2	0.0025	0.99
	Harvest (H)	3	0.16	0.87
	S x H	6	0.78	0.38
	Error	24	0.70	
Gram -	Stand Type (S)	2	0.54	0.81
	Harvest (H)	3	0.38	0.93
	S x H	6	4.74	0.12
	Error	24	2.46	
Actinomycetes	Stand Type (S)	2	0.58	**0.0002**
	Harvest (H)	3	0.034	0.56
	S x H	6	0.085	0.15
	Error	24	0.048	
16:1ω5	Stand Type (S)	2	0.38	**0.036**
	Harvest (H)	3	0.45	**0.012**
	S x H	6	0.033	0.91
	Error	24	0.099	
Fungi/bacteria	Stand Type (S)	2	0.00033	0.96
	Harvest (H)	3	0.0030	0.81
	S x H	6	0.011	0.38
	Error	24	0.0093	
Basal Respiration	Stand Type (S)	2	346.4	**0.029**
	Harvest (H)	2	196.4	0.11
	S x H	4	132.4	0.20
	Error	18	79.8	

Note: Bold-faced values indicate a significant effect at $P<0.05$. df = degrees of freedom

Table 4-5. PLFA biomarker levels in FH-layer forest floors from SPRUCE, MIXED and ASPEN clearcuts (0%), and 20% retention, 50% retention and uncut control (100%) treatments.

	SPRUCE				MIXED				ASPEN			
	0%	20%	50%	100%	0%	20%	50%	100%	0%	20%	50%	100%
Fungi	13.3	15.8	17.9	16.6	16.1	17.3	16.5	14.8	17.0	16.3	15.6	16.4
(mole %)	(0.7)	(0.6)	(1.0)	(3.6)	(1.0)	(0.6)	(1.0)	(1.5)	(1.1)	(1.6)	(1.2)	(1.4)
Bacteria	37.3	35.8	34.3	34.6	35.9	34.6	35.6	36.6	33.6	36.1	36.0	36.0
(mole %)	(0.7)	(0.7)	(0.6)	(1.5)	(0.7)	(1.5)	(0.4)	(1.5)	(0.6)	(1.4)	(1.5)	(0.4)
Gram +	14.8	14.0	13.3	13.5	13.9	14.0	13.9	13.7	13.4	13.9	13.9	14.3
(mole %)	(0.3)	(0.1)	(0.3)	(0.9)	(0.1)	(0.5)	(0.2)	(0.6)	(0.4)	(0.5)	(0.8)	(0.1)
Gram –	25.1	24.1	23.2	23.0	25.2	22.8	24.2	24.9	22.4	24.4	24.9	24.2
(mole %)	(0.4)	(0.5)	(0.6)	(1.1)	(0.9)	(1.8)	(0.3)	(1.2)	(0.7)	(0.7)	(0.9)	(0.3)
Fungi/bacteria	0.36	0.45	0.53	0.49	0.45	0.51	0.47	0.41	0.51	0.46	0.44	0.46
	(0.03)	(0.03)	(0.04)	(0.13)	(0.04)	(0.03)	(0.03)	(0.06)	(0.04)	(0.07)	(0.05)	(0.04)

Note: Values are means (n=3) with standard error in parentheses.

Table 4-6. Indices of microbial community structure calculated using PLFA and SIR data from FH-layer forest floors from SPRUCE, MIXED and ASPEN clearcuts (0%), and 20% retention, 50% retention and uncut control (100%) treatments.

	SPRUCE				MIXED				ASPEN			
	0%	20%	50%	100%	0%	20%	50%	100%	0%	20%	50%	100%
PLFA Diversity	1.78 (0.01)	1.75 (0.02)	1.74 (0.01)	1.77 (0.02)	1.73 (0.02)	1.77 (0.01)	1.75 (0.01)	1.75 (0.01)	1.72 (0.02)	1.72 (0.02)	1.71 (0.01)	1.72 (0.01)
PLFA Richness	52.3 (0.6)	45.9 (2.2)	49.5 (2.3)	50.9 (1.5)	48.3 (1.9)	49.3 (2.1)	49.7 (0.2)	48.6 (2.7)	53.8 (1.4)	47.6 (5.0)	50.1 (2.7)	50.1 (2.5)
PLFA Evenness	0.45 (0.001)	0.46 (0.002)	0.45 (0.01)	0.45 (0.004)	0.45 (0.003)	0.46 (0.01)	0.45 (0.003)	0.45 (0.005)	0.43 (0.002)	0.45 (0.01)	0.44 (0.009)	0.44 (0.004)
Catabolic Evenness	19.4 (0.5)		19.0 (0.5)	18.8 (0.05)	19.1 (0.5)		18.6 (0.5)	18.5 (0.5)	20.0 (0.1)		20.0 (0.2)	19.8 (0.08)

Note: Catabolic evenness was not assessed in FH-layer forest floors from 20% retention treatments. Values are means (n=3) with standard error in parentheses.

Table 4-7. Results of analyses of variance examining the effects of stand type and harvesting treatments on indices of microbial community structure calculated using PLFA and SIR data from FH-layer forest floors.

Variable	Factor	df	MS	*P*
PLFA Diversity	Stand Type (S)	2	0.0057	**0.0014**
	Harvest (H)	3	0.00032	0.69
	S x H	6	0.00080	0.33
	Error	24	0.00065	
PLFA Richness	Stand Type (S)	2	5.91	0.71
	Harvest (H)	3	22.69	0.29
	S x H	6	10.50	0.72
	Error	24	17.08	
PLFA Evenness	Stand Type (S)	2	0.00057	**0.036**
	Harvest (H)	3	0.00030	0.14
	S x H	6	0.000019	0.99
	Error	24	0.00015	
Catabolic evenness	Stand Type (S)	2	3.3	**0.0043**
	Harvest (H)	2	0.5	0.34
	S x H	4	0.08	0.95
	Error	18	0.4	

Note: Bold-faced values indicate a significant effect at $P<0.05$. df = degrees of freedom

Table 4-8. Indicator values of specific PLFAs associated with FH-layer forest floors from ASPEN, MIXED and SPRUCE stands.

	Observed indicator values			Indicator value from randomized tests	
	ASPEN	MIXED	SPRUCE	Mean	*P*
a14:0	53	3	0	20.0 (6.7)	0.0010
11:0 2OH	0	2	65	19.7 (6.3)	0.0010
15:1ω8c	0	24	54	26.3 (6.1)	0.0010
16:1ω9c	0	22	49	26.3 (5.8)	0.0020
17:1ω7c	0	15	43	22.2 (6.6)	0.0080
a19:0	0	34	50	27.7 (5.9)	0.0050

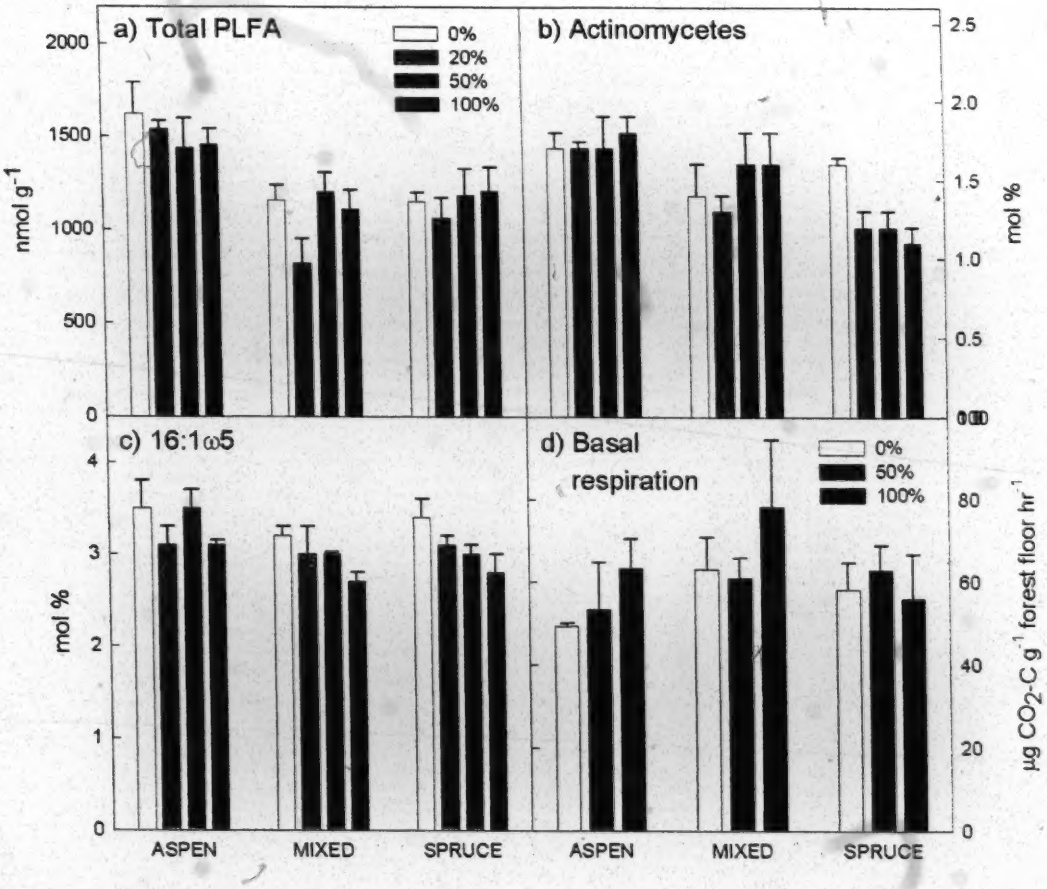

Figure 4-1. Concentrations of total PLFAs, proportions of actinomycetes and the PLFA 16:1ω5, and basal respiration rates in FH-layer forest floors from SPRUCE, MIXED and ASPEN clearcuts (0%), and 20% retention, 50% retention and uncut control (100%) treatments

Note: Values are means (n=3). Error bars represent one standard error.

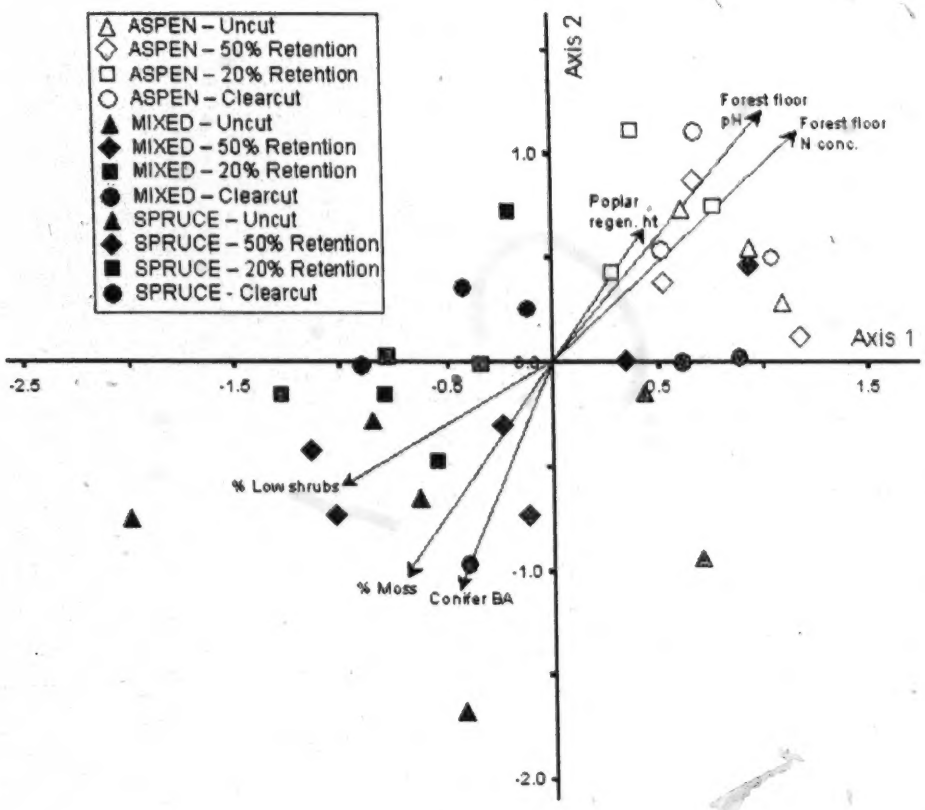

Figure 4-2. NMS ordination biplot of the PLFA profiles of forest floors from ASPEN, MIXED and SPRUCE stands.
Note: NMS ordination produced a solution with a stress of 12.05, which was achieved after 70 iterations.

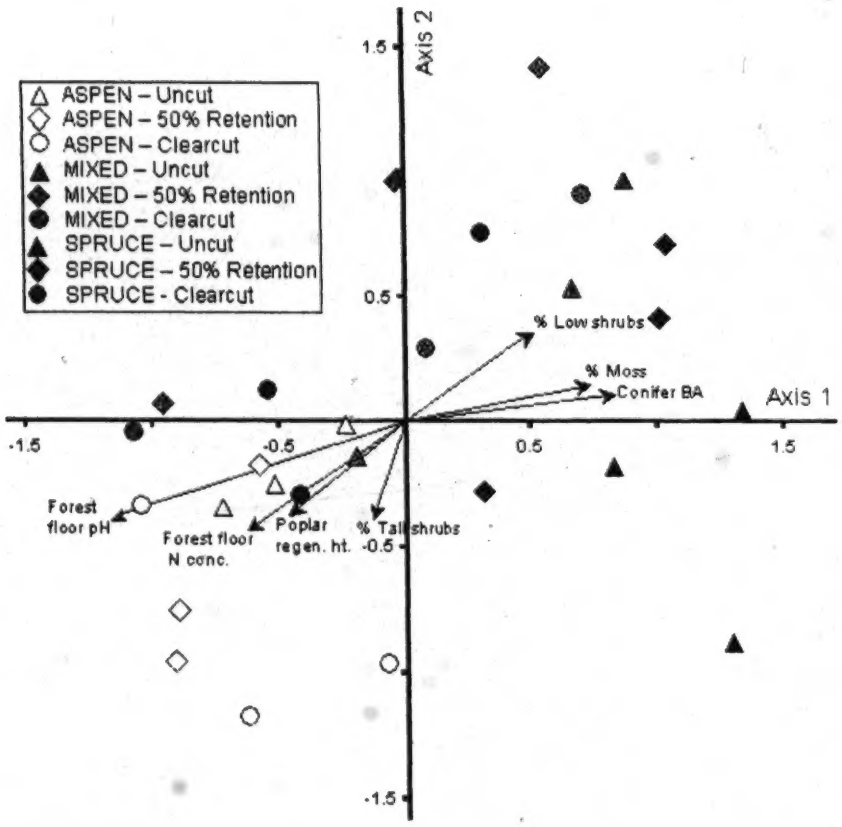

Figure 4-3. NMS ordination biplot of the SIR profiles of forest floors from ASPEN, MIXED and SPRUCE stands.
Note: NMS ordination produced a solution with a stress of 12.79, which was achieved after 54 iterations

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Chapter 5 The forest floor microbial community is resistant to alterations in soil microclimate and inputs from above- and belowground in stands dominated by white spruce or trembling aspen

Introduction

The results of numerous studies indicate that the structure of the soil microbial community varies strongly with the composition of the forest plant community (e.g. Priha et al., 2001; Leckie et al., 2004; Grayston and Prescott, 2005). There are several possible explanations for such a pattern. For example, inter-species variation in the quantity and quality of root exudates can result in the development of distinct rhizosphere microbial communities (Grayston and Campbell, 1996; Griffiths et al., 1999; Kuske et al., 2002). Litter inputs from aboveground can affect soil microbial community structure by altering carbon availability (Schutter and Dick, 2001), nitrogen availability (Broughton and Gross, 2000; Allison et al., 2005), soil organic matter composition (Saetre and Bååth, 2000) and soil pH (Priha et al., 2001). Given that soil temperature and moisture conditions can affect microbial community structure (Zogg et al., 1997; Zak et al., 1999; Fierer et al., 2003), differences among plant species in canopy morphology and phenology may also influence soil microbial communities via altered thermal, moisture and light regimes at the soil surface (Saetre and Bååth, 2000; Priha et al., 2001). In all likelihood, the quantity and quality of inputs from above- and belowground, as well as soil moisture and temperature regimes, are all important factors leading to the development of soil microbial communities that reflect the composition of the forest vegetation.

Reciprocal transfer studies have been employed since the early 1980s to examine the causes of altered soil properties following disturbance, or to better understand the influence of vegetation and climate on soil properties (Table 5-1). These studies generally involve incubations of soils contained in polyethylene bags or resin cores in the site of origin and in another site of differing disturbance history, elevation, vegetation composition etc.. However, enclosure in non-porous plastic bags or resin-capped plastic tubes prevents or inhibits fluctuations in soil moisture, movement of soil fauna, inputs of litter and throughfall and in-growth of fine roots and mycorrhizal hyphae, all of which

can be expected to vary among sites of differing climate, vegetation or disturbance history. More recently, soils have been incubated in mesh-capped plastic tubes or, even better, in mesh cages that may allow more natural fluctuations in soil moisture, soil biota and inputs from above- and belowground (Clein and Schimel, 1995; Bottomley et al., 2004; Balser and Firestone, 2005). Although most reciprocal transfer studies have been employed to examine the dynamics of nitrogen, phosphorus or carbon cycles (Matson and Vitousek, 1981; Jonasson et al., 1993; Clein and Schimel, 1995; Hart and Perry, 1999; Prescott et al., 2003), more recent studies have used reciprocal transfer experiments to examine the effects of climate and vegetation on the composition of the soil microbial community (Bottomley et al., 2004; Balser and Firestone, 2005).

The western boreal mixedwood forest is characterised as a mosaic of forest types, and includes stands dominated by deciduous species, particularly trembling aspen (*Populus tremuloides* Michx.), stands dominated by coniferous species, particularly white spruce (*Picea glauca* (Moench) Voss), and stands with varying proportions of coniferous and deciduous trees. Previous studies have shown that forest floors from ASPEN stands typically support a greater microbial biomass with a different microbial community structure than forest floors from SPRUCE stands (Flanagan and Van Cleve, 1983; Lindo and Visser, 2003; Hannam et al., submitted) but it remains unclear what variables drive this pattern. In this study, a modified reciprocal transfer technique, using forest floors that had developed beneath a trembling aspen or a white spruce canopy, was employed to examine some of the factors that may influence microbial biomass and microbial community structure in forest floors from ASPEN and SPRUCE stands. The quantity, quality and timing of inputs from belowground were manipulated by incubating the forest floors in coarse or fine mesh bags that allowed or excluded fine root in-growth. The quantity, quality and timing of inputs from aboveground (e.g., litter inputs or throughfall), as well as soil microclimate, were manipulated by incubating the forest floors in either ASPEN or SPRUCE stands.

Materials and Methods

Study site

The EMEND site (56° 46' 13" N, 118° 22' 28" W) is located on the boreal mixedwood plain in the Clear Hills Upland Ecoregion within the Boreal Plains EcoZone (Wiken, 1986; EcoRegions Working Group, 1989). The area is characterized by cold winters (mean temperature -14.0°C), warm summers (mean temperature 11.6°C), and an average 433 mm of precipitation, 2/3 of which usually falls during the summer (Environment Canada, 2004). The site has a rolling topography ranging in elevation from 677 to 880 m asl. Soils are usually Brunisols, Orthic Gray Luvisols or Dark Gray Luvisols that have developed on fine-textured glacio-lacustrine or glacial till parent materials (Kishchuk, 2004).

Within the EMEND site are stands dominated by conifers, especially white spruce (SPRUCE), stands dominated by deciduous species, especially trembling aspen (ASPEN), and stands that support approximately equal proportions of both coniferous and deciduous species (MIXED). Stand age ranges from 70 to 130 years (Spence and Volney, 1999). The age of MIXED stands is usually considered intermediate between younger ASPEN stands and older SPRUCE stands.

Mesh bag preparation

In June 2003, bulk samples of forest floor were collected from within clumps of trembling aspen or white spruce growing in a MIXED stand (to ensure that the two forest floor types were of similar age). Beneath trembling aspen, forest floors most closely resembled mormoders and beneath white spruce, forest floors most closely resembled humimors (Green et al., 1993). During collection, litter was removed from the surface of the forest floor and FH-layer forest floor materials from beneath aspen or spruce trees were placed in separate plastic bags. Upon return to the laboratory, bulk ASPEN and SPRUCE forest floors were sieved (4.7 mm) to remove larger roots and twigs, thoroughly homogenized and stored at 5°C.

Subsamples of bulk ASPEN and SPRUCE forest floors were removed in order to determine pre-incubation levels of forest floor pH and concentrations of total C, total N, mineral N, soluble organic C and microbial C. Additional subsamples were frozen at –86°C for subsequent assessment of microbial community structure using phospholipid

fatty acid (PLFA) analysis. Table 5-2 describes the characteristics of the ASPEN and SPRUCE forest floors prior to incubation in the mesh bags.

Open-topped bags (5 cm diameter by 10 cm height, 196 cm^3 volume) of two mesh sizes were constructed to contain the forest floors during incubation in the field. Each coarse mesh bag consisted of a tubular wall constructed from 20 mm polypropylene mesh, to allow fine root in-growth (Clein and Schimel, 1995), and a floor of 5 mm polypropylene mesh. The walls and floor of fine mesh bags were constructed from 23 μm nylon mesh to exclude fine root, but not mycorrhizal, in-growth (Hodge et al., 2001). The bags were kept open at the top to facilitate the movement of water, from precipitation and throughfall, through the forest floor contained in each bag, and to allow access for fauna moving through the litter layer. Mesh bags were filled with sieved, homogenized ASPEN or SPRUCE forest floor at the approximate density of undisturbed forest floors, and stored at 5°C.

Experimental design

The experiment was designed as a reciprocal transplant. Eight replicate bags of each forest floor origin (ASPEN or SPRUCE) x mesh size (coarse or fine mesh) combination were placed in a 1-m grid pattern in three ASPEN stands and three SPRUCE stands at EMEND (total number of mesh bags in each stand = 32). Bags were placed in the forest floor in early August 2003 by removing the litter layer, excavating a hole the size of the mesh bag, placing the mesh bag into the hole (ensuring contact of the walls of the bag with the forest floor outside the bag), and replacing the litter layer on top of the bag. The bags were collected in early August 2004, after one year of incubation in the field, and transported in coolers to the laboratory, where they were stored at 5°C until samples were processed.

Chemical and biological analyses

Within one week of collection from the field, one mesh bag of each forest floor origin x mesh size combination from all six stands was randomly selected for determination of fine root in-growth (total of 24 mesh bags). The roots from each of the selected bags were carefully removed by hand, gently rinsed with distilled water, dried at 65°C to a constant mass and weighed (Bennett et al. 2002). All roots were less than 2 mm in diameter.

The forest floors contained in the seven remaining bags of each treatment combination were sieved (4.7 mm) to remove fine roots, and composited in preparation for all subsequent analyses. Forest floor moisture content was determined gravimetrically by drying at 65°C. Forest floor pH was estimated using a glass electrode pH meter after preparing slurries of forest floor in 0.01 M $CaCl_2$ at a ratio of 1:10 forest floor (fresh weight):solution. Extractable nitrate and ammonium concentrations were determined in 1 M KCl solution using a using a Technicon Auto Analyzer II (Technicon Industrial Systems, Tarrytown, New York). Soluble organic nitrogen (SON) and soluble organic carbon (SOC) concentrations were determined after extraction in 0.5 M K_2SO_4 solution, using a Shimadzu TOC-VTN instrument (Mandel Scientific Company Inc., ON, Canada). Microbial biomass C and N were determined using the chloroform-fumigation extraction method as per Horwath and Paul (1994), except that no correction factors were used in the final calculations of biomass C and N.

Soil fauna were extracted over a period of 10 days from subsamples of forest floor weighing approximately 20 g using a Macfadyen extractor, in which a temperature and moisture gradient was used to force fauna to move downward (Macfadyen, 1961) into an ethylene glycol preservative. Fauna were filtered from the ethylene glycol and stored in 70% ethanol solution. Abundances of oribatid, prostigmatid and mesostigmatid mites, as well as Collembola, were determined under a dissecting microscope.

Aliquots of forest floor samples were frozen at -86°C and then freeze-dried for PLFA analysis. Polar lipids were extracted from 300 mg of freeze-dried forest floor material using a modified Bligh and Dyer extraction (Bligh and Dyer, 1959; Frostegård et al., 1991; White and Ringelberg, 1998). Polar lipid extracts were purified on pre-packed silicic acid columns (Agilent Technologies, Wilmington, DE) and subjected to mild alkaline methanolysis to form fatty acid methyl esters (FAMEs). FAMEs were separated and quantified using an Agilent 6890 Series capillary gas chromatograph (Agilent Technologies, Wilmington, DE) equipped with a 25 m Ultra 2 (5%-phenyl)-methylpolysiloxane column. Hydrogen was used as the carrier gas. Peaks were identified using bacterial fatty acid standards and MIDI peak identification software (MIDI, Inc., Newark, DE).

Fatty acids were designated X:YωZ, where X represents the number of carbon atoms, Y represents the number of double bonds, and Z indicates the position of the first double bond from the aliphatic (ω) end of the molecule. Prefixes 'i' and 'a' indicate branching at the second or third carbon atom, respectively, from the ω end. The prefix 'cy' indicates the presence of a cyclopropyl group somewhere along the carbon chain, while '10Me' indicates a methyl group at the tenth carbon atom from the carboxyl end of the molecule. Cis or trans configurations are indicated by the suffixes 'c' or 't', respectively. Only fatty acids of less than 20 carbons in chain length were included in the calculation of total PLFAs.

Total phospholipid fatty acids were compared between forest floor origins, stand types during incubation and mesh sizes as nmol g^{-1} forest floor, while PLFAs used as biomarkers (Table 5-3; Frostegård and Bååth, 1996; Hassett and Zak, 2005) were compared on a mole % basis in order to standardize for differences in the total amount of PLFAs in forest floors of ASPEN or SPRUCE origin. Indices of community structure, including richness, diversity and evenness were also calculated (Schutter and Dick, 2001).

SIR profiles of the FH-layer forest floor microbial community were obtained using a modified version of the technique developed by Degens and Harris (1997). Because of insufficient forest floor material to complete the analyses on 24 samples from individual replicates, forest floors were combined by treatment into 8 composite samples. Composited samples were brought to equivalent moisture contents (3.9 mL water g^{-1} forest floor) and stored at approximately 5 °C for two weeks. Twenty carbon compounds were selected as test substrates based on their ability to discriminate between treatments in previous studies (Degens and Harris, 1997) and their availability in the laboratory (Table 5-4). The day before analysis, duplicate 1 g (dry weight equivalent) aliquots of forest floor were dispensed into 140 mL glass vials and sealed with Parafilm. Solutions containing the test substrates were brought to the desired concentration ((Degens and Vojvodić-Vuković, 1999; Table 5-4) after adjusting solution pH to between 6.5 and 7.0 using concentrated NaOH or HCl (Degens, 1998) Catabolic response profiles were determined by measuring the carbon dioxide respired by the forest floor in each vial after the addition of 1 mL of distilled deionised water (as a control) or 1 mL of a solution

containing a test substrate, which raised the moisture of the forest floor in each vial to 4.9 mL g^{-1}, or approximately 60% water-holding capacity. Immediately after addition of the solution, each vial was sealed with a rubber septum, vortexed, and incubated in a water bath at 25°C for one hour. Samples were vortexed a second time immediately prior to analysis. Head-space carbon dioxide concentrations were determined using a Hewlett Packard 5890 Series II gas chromatograph equipped with a 1m Poropak Q column, a HP3396 Series II integrator and helium as the carrier gas. A one-hour incubation was employed in this study because data from a pre-trial indicated that large changes in microbial biomass occurred when similar forest floor samples were incubated for more than an hour (data not shown).

Data analysis

Data were analysed as a three-way (forest floor origin x stand-type during incubation x mesh size) factorial ANOVA. Most of the data did not require transformation to meet the assumptions of the analyses. However, there were many zero-values in the fine root and nitrate data, and transformations were not capable of obtaining either a normal distribution or homogeneity of variance. Therefore, the fine root and nitrate data are presented, but ANOVAs were not performed. Basal respiration rates were determined on composited samples. Consequently, basal respiration rates and estimates of qCO_2, calculated as the ratio of basal respiration (μg CO_2-C g^{-1} forest floor $hour^{-1}$) to total PLFAs (nmol g^{-1} forest floor; Balser and Firestone, 2005), were also not compared statistically. For all analyses that were possible, differences were considered statistically significant if $P<0.05$. Pearson correlations were calculated between PLFA biomarkers, concentrations of mesofauna and forest floor chemical properties but the results were not enlightening and, therefore, are not reported. All analyses were performed using SAS (version 8.01, SAS Institute Inc. 1999-2000, Cary, NC).

Patterns in the mole % of individual forest floor PLFAs (after ranking) among forest floor origins, stand-types during incubation, and mesh sizes were examined with the non-metric multidimensional scaling (NMS) ordination technique (Kruskal, 1964; Mather, 1976) using PC-ORD software (version 4, MjM Software Design, Gleneden Beach, OR). The Sorensen (Bray-Curtis) distance measure was used in the analyses. The multiple response permutations procedure (MRPP) was used to compare the distances in the

ordination space between points corresponding to different forest floor origins, stand types during incubation, and mesh sizes in order to determine whether these groups were statistically different. Bonferroni corrections were used to determine a family error rate for the MRPP analyses (Legendre and Legendre, 1998).

Patterns of SIR in response to the addition of the 20 individual test substrates (after standardization; Degens et al., 2001) were also compared between forest floor origins, stand-types during incubation, and mesh sizes using the non-metric multidimensional scaling (NMS) ordination technique (Kruskal, 1964; Mather, 1976). Because these analyses were determined using subsamples from composited forest floors, statistical comparisons of treatment effects were not possible and MRPP was not employed.

Results

One year of incubation in the field was sufficient for numerous roots to grow into the forest floors contained within the coarse mesh bags (Figure 5-1); the mean dry mass of fine roots in coarse mesh bags ranged from approximately 0.14 g in ASPEN stands to approximately 0.041 g in SPRUCE stands. Root in-growth was completely prevented by incubation in fine mesh bags. Overall, mesh size did not have a significant effect on the moisture content of the forest floors contained within the mesh bags (P=0.12; Figure 5-1). However, in ASPEN stands, ASPEN forest floors contained within coarse mesh bags tended to be dryer than ASPEN forest floors contained within fine mesh bags. This pattern was reflected in a significant interaction between stand type and mesh size effects on forest floor moisture content (P=0.034).

Forest floor microbial community composition

The mean concentration of total PLFAs ranged from approximately 400 nmol g^{-1} forest floor to 800 nmol g^{-1} forest floor (Figure 5-2). Forest floors of ASPEN origin contained significantly higher concentrations of total PLFAs (P=0.017) and a greater relative abundance of bacteria (P=0.0032) than forest floors of SPRUCE origin. There were no significant effects of the stand-type during incubation or the mesh size of the bags on total PLFA concentrations or the relative abundance of fungi, bacteria, Gram + or Gram – bacteria, or the ratio of fungal/bacterial PLFAs (Figure 5-2). There were also no significant differences in the indices of community structure (richness, evenness or

Shannon diversity) between forest floor origins, stand-types during incubation or mesh sizes (data not shown).

In all, 49 PLFAs were used in the NMS analysis. The distribution of PLFAs in forest floors of ASPEN origin differed significantly from the distribution of PLFAs in forest floors of SPRUCE origin (Figure 5-3; A=0.080, P=0.011). There was no difference in the distribution of PLFAs in forest floors incubated in ASPEN or SPRUCE stands (A=-0.00048, P=0.36) or in the pattern of PLFAs incubated in fine or coarse mesh bags (A=-0.012, P=0.62), even when forest floors of ASPEN and SPRUCE origin were analyzed separately (data not shown). The family error rate, obtained using the Bonferroni correction, was P<0.017.

Surprisingly, forest floors of SPRUCE origin tended to have greater rates of basal respiration than forest floors of ASPEN origin (although this could not be assessed statistically because analyses were performed on composited samples; Figure 5-4). This pattern is opposite to that expected (Flanagan and Van Cleve, 1983; Lindo and Visser, 2003) and may be due to the fact that ASPEN and SPRUCE forest floors were collected from small clumps of trembling aspen or white spruce in MIXED stands, where forest floor microbial activity has not been well-studied. However, estimates of qCO_2 did not differ strongly between forest floors of ASPEN and SPRUCE origin (Figure 5-4), which suggests that C availability was probably similar in the two forest floor types. The NMS ordination of the SIR data also indicated that the origin of the forest floor had the strongest effect on the metabolic activity of these microbial communities (Figure 5-5). Forest floors of ASPEN and SPRUCE origin tended to separate along axis 1.

Forest floor chemical characteristics

Mean ammonium concentrations ranged from approximately 60 $\mu g\ g^{-1}$ forest floor to 140 $\mu g\ g^{-1}$ forest floor, while mean nitrate concentrations ranged from zero to approximately 13 $\mu g\ g^{-1}$ forest floor (Table 5-5). Neither the origin of the forest floor, the stand-type during incubation, nor the mesh size of the bags had any effect on the concentrations of extractable ammonium in the forest floor. However, there was a strong apparent effect of the stand-type during incubation on the concentration of nitrate in these forest floors (although differences were not tested statistically). Nitrate was frequently detected in forest floors that had been incubated in ASPEN stands but was never detected

in forest floors that had been incubated in SPRUCE stands, regardless of where the forest floor originated or whether fine root in-growth was allowed.

Concentrations of SOC, microbial biomass C and microbial biomass N were all significantly greater in forest floors of ASPEN origin than in forest floors of SPRUCE origin (P=0.0004, P=0.0044, P<0.0001, respectively). There was also a strong trend toward a greater concentration of SON in forest floors of ASPEN origin than in forest floors of SPRUCE origin (P=0.056). However, neither the stand-type during incubation nor the mesh size of the bags had an effect on the concentrations of SOC, SON, microbial biomass C or microbial biomass N in these forest floors (Table 5-5).

Mean forest floor pH ranged from approximately 5.1 to 6.3 (Table 5-5). The pH of forest floors of ASPEN origin was significantly higher than the pH of forest floors of SPRUCE origin (P<0.0001). Furthermore, the pH of forest floors incubated in coarse mesh bags, where fine root in-growth was allowed, was consistently lower than that of forest floors incubated in fine mesh bags, where fine roots were excluded (P=0.0003). However, the stand-type during incubation had no effect on forest floor pH.

Forest floor mesofaunal populations

The mean total number of forest floor-dwelling mesofauna ranged from approximately 5 to 20 individuals g^{-1} forest floor (Figure 5-6). Prostigmatid mites were significantly more abundant (P=0.0050) in forest floors of ASPEN origin than in forest floors of SPRUCE origin. Furthermore, oribatid mites, prostigmatid mites and total mesofauna were significantly less abundant in forest floors incubated in fine mesh bags than in coarse mesh bags (P=0.0007, P=0.0022, P<0.0001, respectively). However, the stand-type during incubation had no effect on the populations of oribatid, mesostigmatid or prostigmatid mites or on the populations of Collembola or total mesofauna. There were significant interactions between the effects of the origin of the forest floor and mesh size on mesostigmatid mites (P=0.0035) and between the effects of stand-type during incubation and the origin of the forest floor on total mesofauna (P=0.017). This latter effect can be seen in Figure 5-6, where forest floors of ASPEN origin have the highest total number of mesofauna when incubated in ASPEN stands while forest floors of SPRUCE origin have the highest total number of mesofauna when incubated in SPRUCE stands.

Discussion

A reciprocal transfer experiment, using coarse and fine mesh bags that allowed or excluded fine root in-growth, was employed to examine how the microbial community composition of forest floors of ASPEN or SPRUCE origin responded to changes in inputs from belowground, inputs from aboveground and microclimatic conditions during a year-long incubation. However, no changes in microbial biomass or microbial community composition were detected, despite treatment differences in moisture content, fine root biomass, pH, nitrate concentrations and mesofauna abundance. Instead, the origin of the forest floor remained the dominant factor controlling forest floor microbiological characteristics.

Previous studies have also demonstrated that the soil microbial community can be somewhat resistant to changes in soil microclimate or litter inputs, despite altered nutrient dynamics. In a 15-month reciprocal transfer experiment using forest floors from an alder and a poplar stand in Alaska, microbial C and N concentrations were constrained by forest floor origin, despite strong treatment effects on C and N mineralization rates (Clein and Schimel, 1995). Twenty-seven months after the transfer of soils from a grassland to a forest, the only detectable change in PLFA biomarkers was an increase in the concentration of fungal biomass, despite significant reductions in N_2O production (Balser and Firestone, 2005). Finally, two years after soils at two sites were transferred from meadows to forests, significant increases in the terminal-restriction fragment *Alu* 390, associated with ammonium-oxidizing bacteria, were observed in soils from one site but no changes were observed at the second site, despite reduced inorganic N concentrations in the transferred soils at both sites (Bottomley et al., 2004). Thus, the activity of the soil microbial community appears to respond more readily to environmental change than does the structure of the soil microbial community.

The historical plant community composition, and its influence on the quantity and quality of soil organic matter, has been found to have a strong effect on soil microbial community characteristics in other disturbed soils (Broughton and Gross, 2000; Degens et al., 2000). Large changes in catabolic evenness, for example, tend to occur only when management practices induce significant reductions in soil organic C concentrations

(Degens et al., 2000; Graham and Haynes, 2005). Microbial communities in C-rich forest floors may be particularly resistant to disturbance because available C is probably depleted over relatively long periods of time or after more intense disturbances. Therefore, one year of incubation may have been insufficient for the effects of forest floor transfer and fine root exclusion to develop in these soils.

Despite the lack of treatment effects on the microbial communities of ASPEN or SPRUCE forest floors in this study, the modified reciprocal transfer technique holds considerable promise for examining the influence of various factors on soil characteristics. Unlike laboratory studies, soils incubated in the field are exposed to more natural light, temperature and moisture regimes and can receive above- and belowground inputs of a similar quantity, quality and timing as those under natural conditions. In contrast to the polyethylene bags or plastic tubes used to contain soils in many previous reciprocal transfer studies, coarse mesh bags allow the in-growth of roots and fungal hyphae, while fine mesh (approximately 20 μm) bags allow the in-growth of fungal hyphae but exclude roots (Hodge et al., 2001). Furthermore, mesh size did not have a strong overall effect on forest floor moisture content in this study, which suggests that the through-flow of soil water may have been similar in coarse and fine mesh bags.

Although there are a number of benefits of using mesh bags in reciprocal transfer studies, there continue to be some difficulties with the reciprocal transfer technique, which must be taken into consideration. As mentioned by Hart and Perry (1999), instantaneous changes in the site of incubation will not accurately capture those ecological effects that require long periods of time to manifest themselves. Therefore, reciprocal transfer techniques, in general, may be more appropriate for examining the influence of such disturbances as timber harvesting, pollution or vegetation change (e.g. Matson and Vitousek, 1981; Prescott et al., 2003), than longer-term effects, such as climate change. Furthermore, incubation in fine mesh bags appears to lower the abundance of soil mesofauna in the forest floor (Figure 5-6). It is not clear whether this pattern is caused by reduced access of soil fauna to forest floors within fine mesh bags or by reduced availability of resources (i.e., roots) for fauna within the bags. Given that soil mesofauna have a strong effect on nutrient cycling dynamics and soil microbial community structure (e.g. Huhta et al., 1988; Edsberg, 2000; Johnson et al., 2005),

changes in soil fauna populations during incubation of reciprocally transferred soils should not be ignored.

Conclusions

In summary, the microbial biomass and microbial community structure (assessed by chloroform fumigation-extraction, by PLFA analysis and by SIR analysis) of forest floors of ASPEN or SPRUCE origin were resistant to changes induced by one year of incubation in ASPEN or SPRUCE stands, with and without fine root in-growth. Despite the lack of changes in microbial community structure, the stand type during incubation had a strong influence on forest floor moisture content and concentrations of nitrate, while mesh size had a significant effect on forest floor pH and the abundance of mesofauna. Therefore, strong changes in microbial community structure were not required in order for changes in nutrient dynamics (i.e., nitrate concentrations) to occur. Further treatment effects may have been detected if the study had been extended beyond one year. The resistence of these forest floor microbial communities to changes in soil microclimate and inputs from above- and belowground may be a function of the high C contents of these forest floors. The modified reciprocal transfer technique employed in this study holds considerable promise for research examining factors that influence soil properties, particularly in the case of disturbances in which site conditions are rapidly altered.

Tables and Figures

Table 5-1. Summary of previous studies using reciprocal transfer techniques to examine factors controlling soil characteristics.

Source	Soil Type	Climate	Sites	Factor(s) under study	Incubation Period	Soil T difference	Incubation Type
Balser & Firestone 2005	Mineral soil	Mediterranean, CA	Forest vs. grassland	Climate & microbial community	27 mo.	5-15 °C, at sampling	Mesh-capped PVC tubes
Bottomley et al. 2004	Mineral soil	Temperate, OR	Forest vs. meadow	Climate & vegetation	2 yr.	unknown	Mesh-capped PVC tubes; mesh cages
Prescott et al. 2003	Forest floor, Mineral soil	High elevation temperate, BC	Forest vs. 5-year-old clearcuts	Clearcutting	6 wk.	2-3 °C	Polyethylene bags
Hart & Perry 1999	Mineral soil	Temperate, OR	High- vs. low-elevation forest	Climate	Summer-5 mo.; Winter-9 mo.	4 °C	Resin cores
Clein & Schimel 1995	Forest floor	Boreal, AK	Poplar vs. alder forest	Vegetation	14 mo.	unknown	Mesh cages
Jonasson et al. 1993	Forest floor	Boreal, Sweden	High-elevation fellfield vs. low-elevation heath	Climate	2 mo.	4-5 °C	Polyethylene bags
Matson & Vitousek 1981	Mineral soil	Temperate, IN	Forest vs. 1-, 3- and 8-year-old clearcuts	Clearcutting	30 d.	0-2 °C	Polyethylene bags

Table 5-2. Initial characteristics of forest floor used in reciprocal transfer study.

	ASPEN origin	SPRUCE origin
C (mg g^{-1})	429.3 (3.2)	454.9 (5.0)
N (mg g^{-1})	15.8 (0.6)	14.8 (0.2)
pH	6.2	5.0
NO_3^- (μg g^{-1})	0.3 (0.4)	0
NH_4^+ (μg g^{-1})	20.1 (0.9)	29.6 (1.5)
SOC (mg g^{-1})	1.9 (0.1)	1.0 (0.03)
Microbial C (mg g^{-1})	4.3 (0.3)	3.8 (0.04)
Total PLFA (nmol g^{-1})	714.5 (7.6)	663.9 (54.0)

Note: Values are means (n=3) with standard error in parentheses.

Table 5-3. PLFAs used as biomarkers

Microbial Group	PLFA Marker
Fungi	18:1ω9c
	18:2ω6 + a18:0
Bacteria	i15:0
	a 15:0
	15:0
	i16:0
	i17:0
	a17:0
	17:0
	cy17:0
	18:1ω7
	cy19:0
Gram +	i15:0
	a15:0
	i16:0
	10Me16:0
	a17:0
	i17:0
Gram –	16:1ω5c
	cy17:0
	cy19:0
	18:1ω5c
	18:1ω7c

Table 5-4. Sole carbon sources used to measure SIR profiles

Type	Concentration (M)	Compound
Amino Acids & Amines	0.015	L-arginine
	0.015	L-asparagine
	0.015	D- (+) -Glucosamine
	0.015	L-glutamic Acid
	0.015	L(-)-histidine
	0.015	DL-lysine
	0.015	DL-serine
Aromatic	0.015	Urocanic Acid
Carbohydrate	0.075	D-glucose
	0.075	D(+)-mannose
	0.075	Sucrose
Carboxylic Acid	0.1	L-ascorbic Acid
	0.1	Citric Acid
	0.1	Fumaric Acid
	0.1	D-gluconic Acid
	0.1	α-ketoglutaric Acid
	0.1	DL-malic Acid
	0.1	Pantothenic Acid
	0.1	Succinic Acid
	0.1	DL-tartaric Acid

Table 5-5. Concentrations of nitrate, ammonium, soluble organic C and N, microbial biomass C and N and pH in forest floors of ASPEN or SPRUCE origin incubated for one year in ASPEN or SPRUCE stands in coarse or fine mesh bags.

		ASPEN origin		SPRUCE origin	
	Stand-type during incubation	Mesh size		Mesh size	
		Coarse	Fine	Coarse	Fine
NO_3^--N (μg/g)	ASPEN	8.8 (7.4)	4.1 (4.1)	12.7 (12.7)	7.4 (4.6)
	SPRUCE	0	0	0	0
NH_4^+-N (μg/g)	ASPEN	58.8 (9.9)	84.9 (20.6)	136.4 (58.0)	70.4 (12.2)
	SPRUCE	70.6 (23.0)	78.8 (5.4)	79.0 (12.6)	69.9 (12.1)
Soluble organic C (mg/g)	ASPEN	2.1 (0.2)	2.3 (0.2.)	1.7 (0.1)	1.6 (0.1)
	SPRUCE	2.2 (0.2)	2.6 (0.3)	1.7 (0.1)	1.8 (0.3)
Soluble organic N (mg/g)	ASPEN	0.14 (0.01)	0.13 (0.02)	0.08 (0.03)	0.11 (0.03)
	SPRUCE	0.13 (0.01)	0.15 (0.02)	0.12 (0.02)	0.13 (0.01)
Microbial C (mg/g)	ASPEN	4.5 (0.3)	4.7 (0.2)	3.5 (0.6)	3.2 (0.7)
	SPRUCE	4.5 (0.3)	5.5 (1.1)	3.4 (0.1)	4.0 (0.3)
Microbial N (mg/g)	ASPEN	0.84 (0.04)	0.88 (0.05)	0.59 (0.09)	0.56 (0.09)
	SPRUCE	0.88 (0.05)	0.87 (0.02)	0.59 (0.02)	0.67 (0.03)
pH	ASPEN	6.0 (0.04)	6.3 (0.08)	5.1 (0.04)	5.3 (0.05)
	SPRUCE	6.1 (0.06)	6.3 (0.01)	5.2 (0.08)	5.3 (0.07)

Note: Values are means (n=3) with standard error in parentheses.

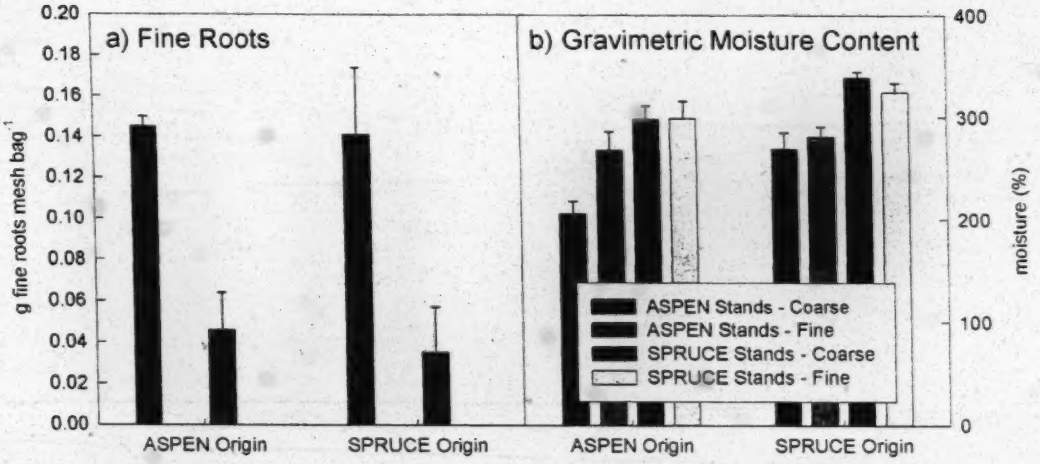

Figure 5-1. Concentrations of fine roots and gravimetric moisture content of forest floors of ASPEN or SPRUCE origin incubated for one year in ASPEN or SPRUCE stands in coarse or fine mesh bags.
Note: Values are means (n=3). Error bars represent one standard error of the mean

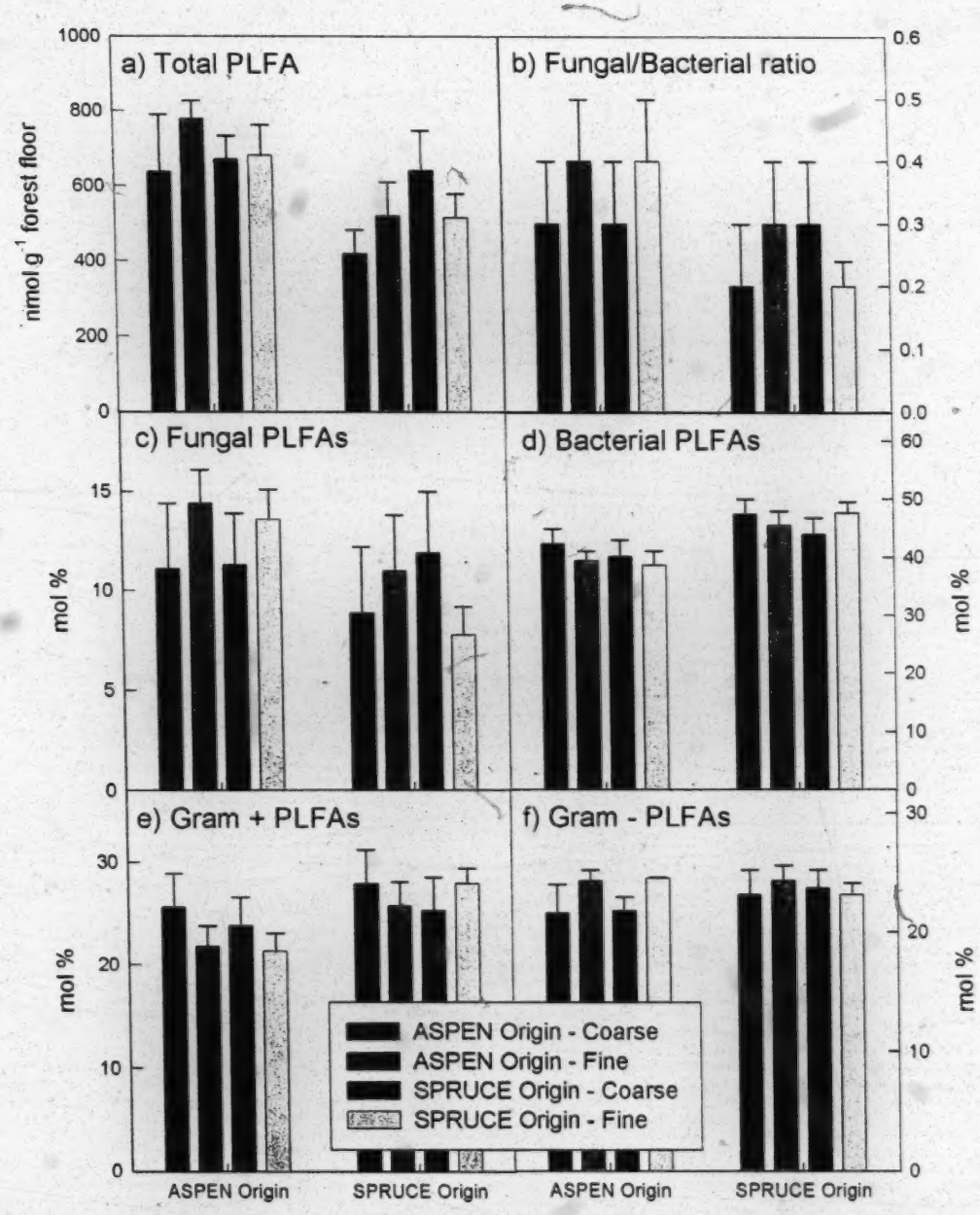

Figure 5-2. Concentrations of total PLFAs, the ratio of fungal/bacterial PLFA biomarkers and the relative abundance of PLFAs associated with fungi, bacteria, Gram + bacteria and Gram – bacteria in forest floors of ASPEN or SPRUCE origin incubated for one year in ASPEN or SPRUCE stands in coarse or fine mesh bags.
Note: Values are means (n=3). Error bars represent one standard error of the mean.

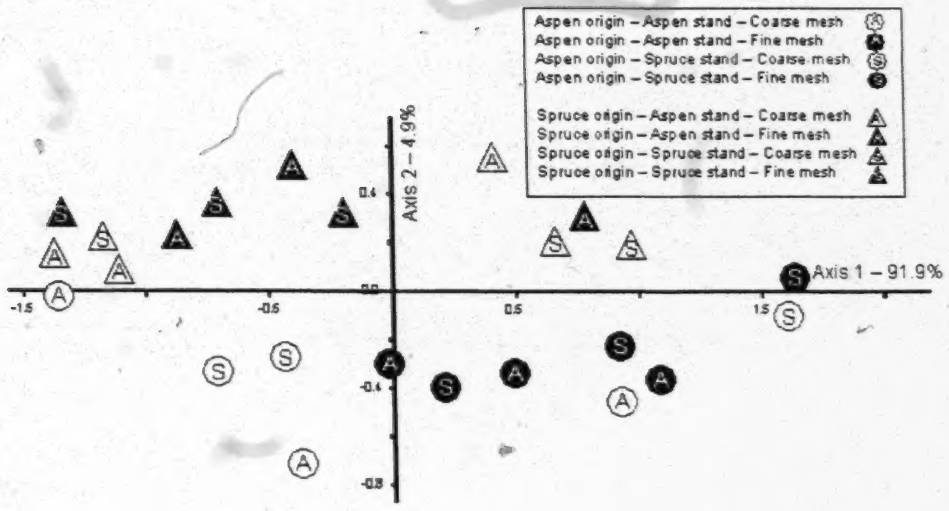
Aspen origin – Aspen stand – Coarse mesh
Aspen origin – Aspen stand – Fine mesh
Aspen origin – Spruce stand – Coarse mesh
Aspen origin – Spruce stand – Fine mesh
Spruce origin – Aspen stand – Coarse mesh
Spruce origin – Aspen stand – Fine mesh
Spruce origin – Spruce stand – Coarse mesh
Spruce origin – Spruce stand – Fine mesh
Axis 2 – 4.9%
Axis 1 – 91.9%
-1.5
-0.5
0.5
1.5
0.4
0.0
-0.4
-0.8

Figure 5-3. NMS ordination biplot of the PLFA profiles of forest floors of ASPEN or SPRUCE origin incubated for one year in ASPEN or SPRUCE stands in coarse or fine mesh bags.
Note: NMS ordination produced a solution with a stress of 6.91, which was achieved after 84 iterations.

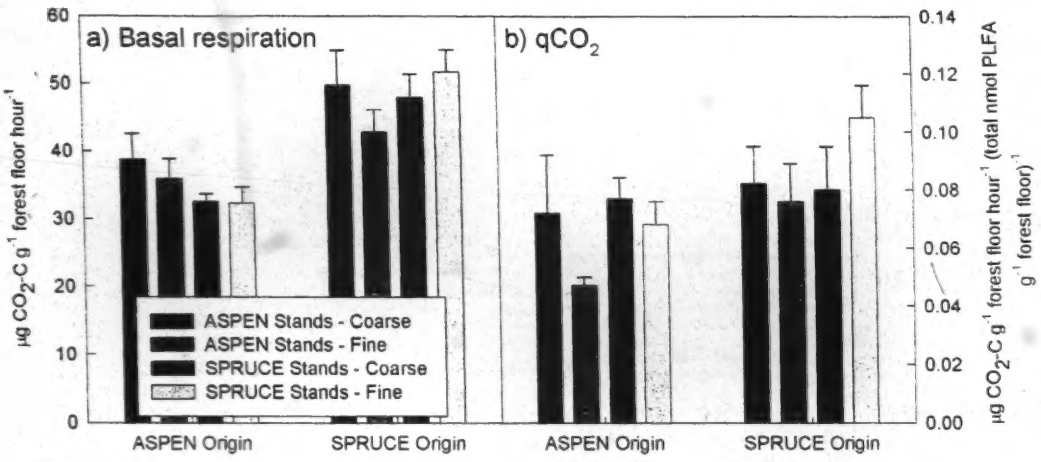

Figure 5-4. Basal respiration rates and qCO_2 values of forest floors of ASPEN or SPRUCE origin incubated for one year in ASPEN or SPRUCE stands in coarse or fine mesh bags.
Note: Data were obtained from composited samples. Values are means (subsamples=2). Error bars represent one standard error of the mean.

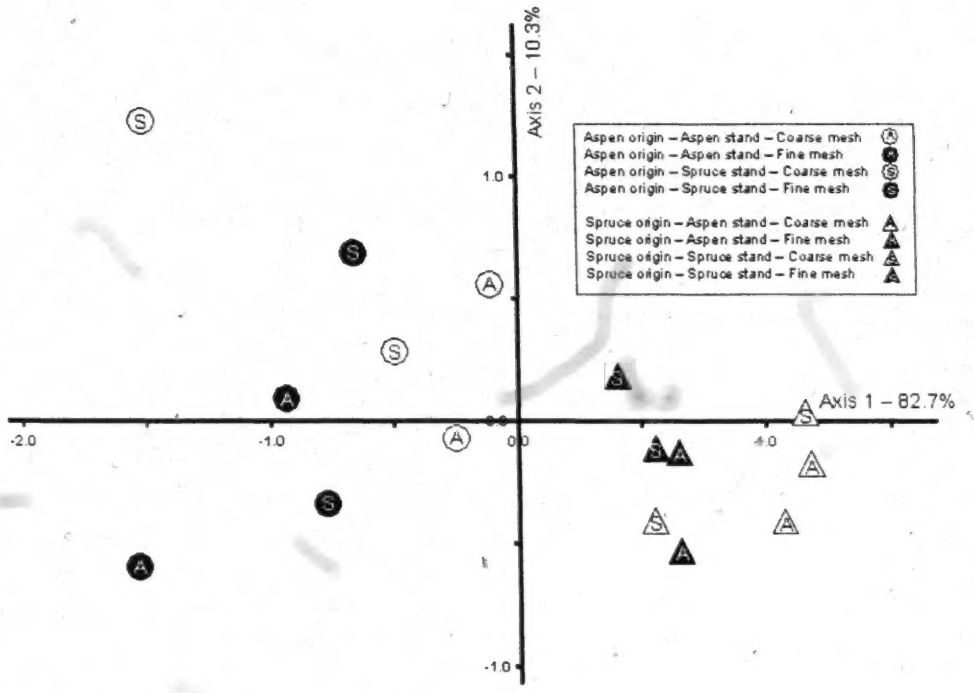
Axis 2 – 10.3%
Axis 1 – 82.7%
Aspen origin – Aspen stand – Coarse mesh
Aspen origin – Aspen stand – Fine mesh
Aspen origin – Spruce stand – Coarse mesh
Aspen origin – Spruce stand – Fine mesh
Spruce origin – Aspen stand – Coarse mesh
Spruce origin – Aspen stand – Fine mesh
Spruce origin – Spruce stand – Coarse mesh
Spruce origin – Spruce stand – Fine mesh
1.0
-1.0
-2.0
-1.0
0.0
4.0

Figure 5-5. NMS ordination biplot of the SIR profiles of forest floors of ASPEN or SPRUCE origin incubated for one year in ASPEN or SPRUCE stands in coarse or fine mesh bags.
Note: Data were obtained from composited samples. NMS ordination of the SIR data produced a solution with a stress of 9.75, which was achieved after 59 iterations.

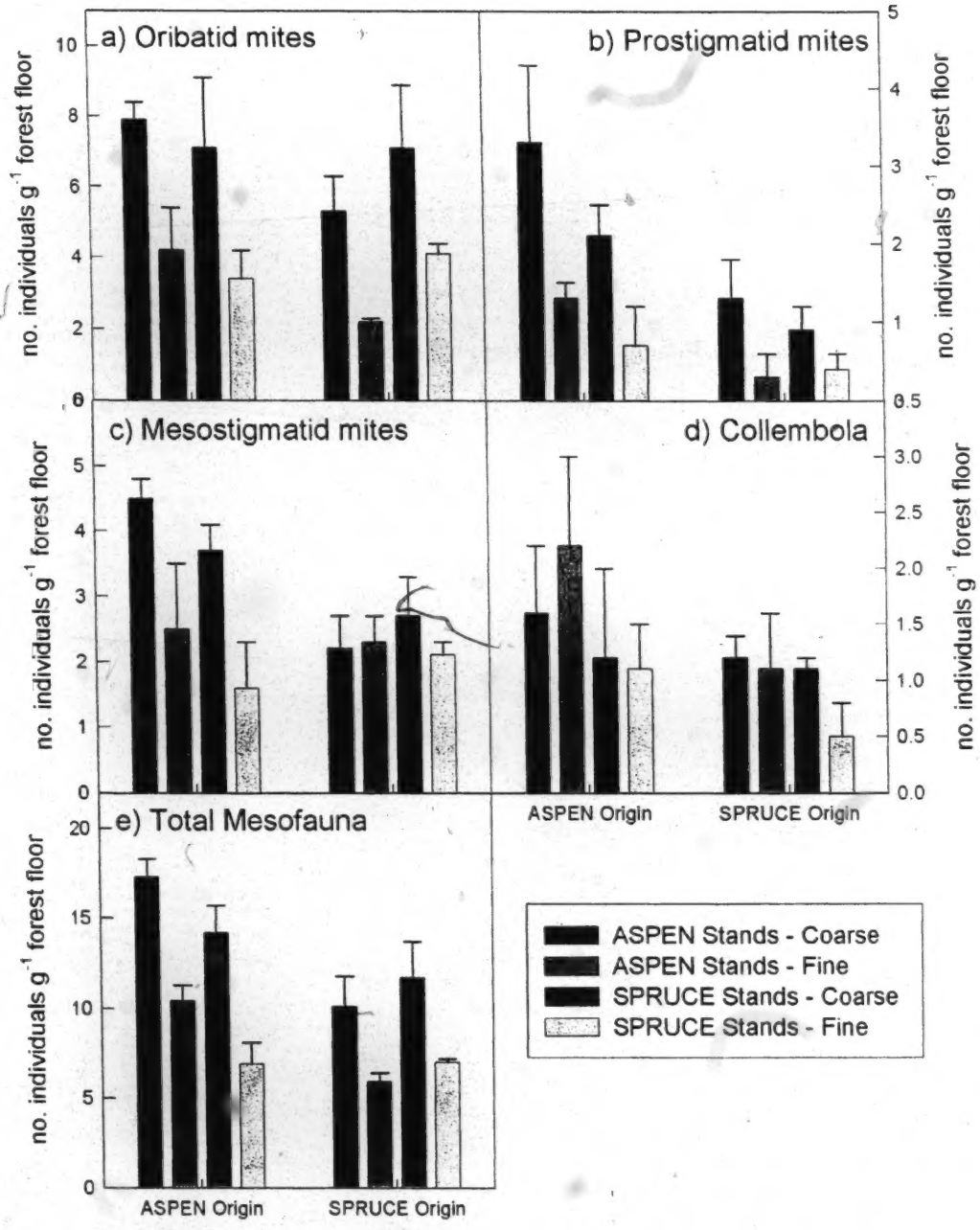

Figure 5-6. Concentrations of oribatid, prostigmatid and mesostigmatid mites, collembola, and total mesofauna in forest floors of ASPEN or SPRUCE origin incubated for one year in ASPEN or SPRUCE stands in coarse or fine mesh bags.
Note: Values are means (n=3). Error bars represent one standard error of the mean.

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Chapter 6 Summary and Conclusions

6.1 Summary

The specific objectives of this research were:

1. To determine how the organic matter composition of forest floors differed between stands dominated by trembling aspen (ASPEN) and stands dominated by white spruce (SPRUCE) at the Ecosystem Management Emulating Natural Disturbance (EMEND) experimental site, and to examine post-harvest changes in the organic matter composition of these forest floors

2. To determine how the microbial community composition of forest floors differed between ASPEN, SPRUCE and mixed-species (MIXED) stands at EMEND, and to examine post-harvest changes in the microbial community composition of these forest floors

3. To examine the influence of belowground inputs, and aboveground inputs and soil microclimate, on the microbial community structure and selected chemical characteristics of ASPEN and SPRUCE forest floors at EMEND.

6.1.1 Forest floor composition in ASPEN and SPRUCE stands

Cross-polarization magic-angle spinning ^{13}C nuclear magnetic resonance spectroscopy (CPMAS ^{13}C NMR) was used to characterize the FH-layer forest floors of ASPEN and SPRUCE stands at EMEND. Small but statistically significant differences were detected between stand types; aromatic carbon content was higher and carbonyl carbon content was lower in the forest floors of SPRUCE stands than in ASPEN stands. Within stand types, correlation analyses indicated significant relationships between the composition of the forest floor and soil temperature, the mass of the litter layer, and the mass of the moss layer. However, these relationships could not explain observed differences in the chemical composition of the forest floor between stand types. Although forest floors from SPRUCE stands were largely composed of moss, which is low in aromatic C, they had a greater aromatic C content than forest floors from ASPEN stands, where moss was rare. Furthermore, a lack of significant correlations across stand types suggested that there may be different relationships between the chemical and environmental characteristics of forest floors from SPRUCE and ASPEN stands.

6.1.2 Forest floor chemical properties are altered by clearcutting in ASPEN and SPRUCE stands

Proximate analysis, ^{13}C isotopic determination and CPMAS ^{13}C NMR spectroscopy were used to examine differences in the characteristics of forest floors from uncut and clearcut SPRUCE and ASPEN stands at EMEND. Proximate analysis revealed no difference in the chemical properties of forest floors from clearcut and uncut stands in either stand type, but the acid-insoluble residue of forest floors from clearcut ASPEN stands was enriched in ^{13}C compared with forest floors from uncut ASPEN stands. CPMAS ^{13}C NMR spectroscopy revealed that forest floors from clearcuts were enriched in total aromatic C, particularly in ASPEN stands, and depleted in phenolic C, particularly in SPRUCE stands. These patterns suggested that forest floors from the clearcuts had become more humified, and may reflect stand-type differences in the amount of labile carbon available to the forest floor microbial community, as well as reductions in above- and below-ground inputs to the forest floor following clearcutting in both stand types. Changes in the chemical properties of forest floors from clearcut SPRUCE and ASPEN stands could exacerbate C limitation in these soils and alter patterns of nutrient cycling.

6.1.3 Forest floor microbial communities in relation to stand composition and timber harvesting

Phospholipid fatty acid (PLFA) and substrate-induced respiration (SIR) analyses were used to compare the microbial biomass and microbial community structure of forest floors from SPRUCE, ASPEN and MIXED stands that had been clearcut, partial-cut with 20% retention, partial-cut with 50% retention or left uncut (controls). Samples of forest floor were collected 4.5 and 5.5 years post-harvest. PLFA and SIR analyses revealed that ASPEN forest floors supported a larger microbial biomass with a very different community structure than MIXED or SPRUCE forest floors. The microbial community structure of these soils appeared to be strongly affected by the presence of white spruce and (or) the composition of the understory vegetation. There were no effects of timber harvesting within or across stand types on any of the variables measured, with the exception of the PLFA 16:1ω5, which was relatively more abundant in the clearcuts and 50% retention treatments than in the uncut controls, perhaps in response to increases in

forest floor pH and grass cover in the disturbed areas. The resilience to timber harvesting of the forest floor microbial communities from these stands may be the result of efforts to minimize soil disturbance during harvesting and to allow vegetation to regenerate naturally. From the perspective of the forest floor microbial community, partial harvesting does not appear to have any benefit over clearcut harvesting at EMEND.

6.1.4 The forest floor microbial community is resistant to alterations in soil microclimate and inputs from above- and belowground in SPRUCE and ASPEN stands

Previous studies have shown that forest floors from ASPEN stands tend to support a greater microbial biomass with a different microbial community structure than forest floors from SPRUCE stands. A reciprocal transfer experiment, in concert with coarse and fine mesh bags that allowed or excluded fine root in-growth, was used to examine how the composition of these forest floor microbial communities respond to differences in belowground inputs from fine roots, aboveground inputs (e.g. from litter and through-fall) and soil microclimatic conditions over one year. Neither the microbial biomass nor the microbial community structure (assessed using PLFA and SIR techniques) of forest floors of ASPEN or SPRUCE origin were altered by reciprocal transfer to SPRUCE or ASPEN stands, with or without fine root inputs. Despite the lack of changes in microbial community structure, the stand type during incubation had a strong effect on forest floor moisture content and concentrations of nitrate, while mesh size had a significant effect on forest floor pH and the abundance of mesofauna. Thus, changes in microbial community structure did not co-occur with changes in other characteristics of these forest floors. The resistance of the forest floor microbial communities to change may be a function of the high C contents of these soils. Further treatment effects may have been detected if the study had been extended beyond one year. Reciprocal transfer studies using coarse and fine mesh bags allow transferred soils to respond to fluctuations in microclimate, organic inputs and soil biota and, therefore, hold considerable promise for studies examining the influence of disturbances on soil properties.

6.2 Project limitations and suggestions for future research

The research projects summarised above addressed the effects of stand type and timber harvesting on the organic matter and microbial community composition of forest floors in the boreal mixedwood forest at EMEND. There are numerous intriguing avenues for future research, including the use of altered sampling regimes, different analytical techniques and the inclusion of forest floors from a wider variety of stand and disturbance types. Some of these are briefly described below.

6.2.1 Altered sampling regime

Prior to the application of the harvesting treatments, the EMEND site was divided into a number of stand-type categories, including ASPEN, MIXED and SPRUCE stands. However, as with most naturally regenerated forests, there is considerable variation in the tree species composition, stand density and understory composition within each of these stand types. In most of the studies described in chapters 2 to 5, samples collected from within each stand were composited prior to sample analysis; this precluded any exploration of relationships, at the within-stand level, between the characteristics of the surrounding vegetation and forest floor composition. Variability in forest floor organic matter composition and forest floor microbial community structure may be more effectively detected, and relationships among forest floor variables and forest vegetation more accurately described by directly relating the chemical and microbial characteristics of each forest floor sample to the vegetation surrounding every sampling point.

As mentioned in previous chapters, the F- and H-layers of the forest floor were sampled and analysed together because the two layers were difficult to separate accurately. However, both microbial community composition (Fritze et al., 2000; Fierer et al., 2003; Leckie et al., 2004) and organic matter composition (Zech et al., 1992; Baldock and Preston, 1995) have been shown to change with depth and forest floor layer or soil horizon. Changes through time in forest floor decay patterns may be better understood if the F- and H-layers of the forest floor were collected and analysed separately. It would also be very interesting to extend sample collection into the mineral soil to determine if the effects of stand type on organic matter and microbial community composition are similar to those in the forest floor. A small study has been initiated, outside of my PhD research, in which the F- and H-layers of forest floors from

undisturbed ASPEN and SPRUCE stands were collected separately, but the results of the NMR analyses of these samples are not yet available.

Previous studies that have examined patterns in microbial community composition at several times over the growing season have found that the structure of the soil microbial community tends to respond readily to seasonal fluctuations in resource availability and soil moisture and temperature conditions (Grayston et al., 2001; Myers et al., 2001; Rogers and Tate, 2001; Krave et al., 2002). Therefore, it would be interesting to monitor forest floor microbial community composition through the growing season to determine if harvesting effects are more evident at certain times.

6.2.2 Modified methods for reciprocal transfer experiment

The use of two sizes of mesh bag was a substantial improvement over the plastic tubes or polyethylene bags used to contain soils in most previous reciprocal transfer studies (e.g., Prescott et al., 2003; Bottomley et al., 2004; Balser and Firestone, 2005). However, there remain a number of improvements that could be made to this type of study if it were to be repeated at EMEND. Given that changes in microbial community composition tend to become stronger with time since reciprocal transplant (Bottomley et al., 2004; Balser and Firestone, 2005), this type of experiment would benefit by at least five years of incubation in the field. In addition, forest floor samples should be collected from the bulk forest floor at the same time that the mesh bags are collected, to verify that enclosure in the bags does not seriously alter forest floor moisture regime, rooting density, abundance of mesofauna etc. Furthermore, it was not possible to separate the effects of altered microclimate from the effects of altered inputs from aboveground, both of which were manipulated simultaneously by incubating the forest floors in ASPEN or in SPRUCE stands. To address this, it may be prudent to include an incubation experiment in the laboratory to examine the influence of altered moisture and temperature regimes on microbial community structure and nutrient cycling dynamics. Alternatively, small dataloggers could be inserted into the forest floors inside or adjacent to the mesh bags so that temperature differences could be accounted for in the data analyses. Finally, net N mineralisation rates and concentrations of mineral N in soils have limited interpretive value (Schimel and Bennett, 2004). Therefore, an assessment of gross processes of N mineralisation, immobilisation and nitrification (Kirkham and

Bartholomew, 1954; Davidson et al., 1991) would be more instructive. It would then be possible to determine, for example, whether nitrate concentrations were elevated in forest floors incubated in the ASPEN stands because nitrate production was higher or because nitrate consumption was lower than in forest floors incubated in SPRUCE stands. These simple changes would improve the utility of the reciprocal transfer technique for examining the factors controlling soil characteristics in the field.

6.2.3 A wider variety of disturbance and stand types

Decomposition and nutrient dynamics are generally believed to be quite different in MIXED stands compared with either ASPEN or SPRUCE stands (Man and Lieffers, 1999), yet this phenomenon has not been intensively studied. The availability of ASPEN, MIXED and SPRUCE stands at EMEND provides the perfect opportunity to address the effects of litter mixing on the patterns of decomposition in forest floors. Another PhD student working at EMEND found that rates of aspen leaf litter decomposition tended to be higher in MIXED stands than in either ASPEN or SPRUCE stands (Lucie Jerabkova, personal communication, The University of British Columbia). To address this, we have sent the trembling aspen leaf litter and white spruce needle litter used in that study for NMR analysis to determine if patterns in litter decay vary with the stand-type in which they are incubated. However, the data from this follow-up study are not yet available.

Finally, one of the main reasons for establishing the EMEND experiment was to compare the effects of various harvesting techniques with those of natural disturbances, particularly wildfire. The effects of timber harvesting and wildfire on the physical and chemical properties of the forest floor have been shown to differ quite widely (Simard et al. 2001). Burning has also been shown to cause stronger changes in forest floor microbial biomass, soil organic matter composition and microbial community structure than timber harvesting, although most previous studies have focused on the effects of post-harvest burns (Entry et al., 1986; Bååth et al., 1995. Pietikäinen and Fritze, 1995; Czimczik et al., 2003). Therefore, it would be very interesting to compare the forest floor organic matter and microbial community compositions of harvested and unharvested stands at EMEND with those of similar stands that have been burned by wildfire.

6.3 Implications of these results for forest management

The results of these studies indicate that conditions in ASPEN stands result in the production of forest floors with an organic matter composition and microbial community structure that is distinct from those in SPRUCE or MIXED stands. There appears to be a strong effect on forest floor characteristics of the presence of white spruce or, perhaps, the understory vegetation associated with white spruce. Therefore, planting white spruce in mixture with trembling aspen on a site that was formerly ASPEN may shift the forest floor characteristics toward those of a SPRUCE stand. Given that forest floor organic matter (Stump and Binkley, 1993; Hart et al., 1994; Prescott et al., 2003) and microbial community composition (Bradley et al., 1997; Priha et al., 1999; Thomas and Prescott, 2000) have the potential to affect nutrient availability, altering the tree species composition of a stand after harvesting has the potential to change nutrient cycling dynamics in regenerating stands over the long term. This could have implications for landscape-scale patterns in productivity and ecological processes across the boreal mixedwood forest.

Neither clearcutting nor partial harvesting had caused a strong change in microbial community structure five years post-harvest at EMEND. Thus, there does not appear to be a benefit, from the perspective of the forest floor microbial community, of utilizing partial harvesting techniques, which can be more time-, cost- and labour-intensive. However, the resilience of these forest floor communities to disturbance may have been due to the relatively careful silvicultural practices employed during the application of the harvesting treatments in this study, e.g. winter-logging, restricting machine traffic to designated corridors, allowing natural regeneration. Thus, it is difficult to know whether the lack of change in forest floor microbial community composition following harvesting is typical of the effects that occur following logging in the boreal mixedwood forest. As a result, further research is required before conclusions can be drawn, with confidence, about the implications of clearcutting and partial harvesting for the organic matter and microbial community composition of forest floors in boreal mixedwood stands.

6.4 Conclusions

In summary, small but significant differences in soil organic matter composition were detected using CPMAS ^{13}C NMR spectroscopy between ASPEN and SPRUCE forest floors. These differences appear to be due to stand type-specific patterns of decomposition and humification. Forest floors from SPRUCE and MIXED stands also supported strongly different microbial community compositions, apparent using both SIR and PLFA techniques, than forest floors from ASPEN stands. The strong and consistent differences in the forest floor microbial community structure of SPRUCE and MIXED stands compared with those of ASPEN stands may be due to the presence of white spruce or, perhaps, the understory vegetation associated with white spruce. However, attempts to determine whether differences in inputs from belowground (e.g., fine roots) or differences in microclimate and inputs from aboveground were more important in controlling microbial community composition were largely unsuccessful, probably because of the short incubation time used and the C-rich status of these forest floors.

Small but statistically significant differences in organic matter composition were detected in the forest floors of ASPEN and SPRUCE clearcuts and uncut stands, but these differences did not translate into significant changes in microbial community composition, measured using SIR or PLFA techniques. The lack of a change in forest floor microbial community structure following harvesting in any of the three stand types was surprising, given that reduced concentrations of microbial biomass and rates of respiration were detected in forest floors following harvesting of ASPEN and SPRUCE stands in an earlier study at EMEND. The microbial communities of these forest floors may be relatively resilient to disturbance, particularly since careful harvesting techniques were employed within the study area.

Taken together, the results of these studies indicate that silvicultural decisions regarding the tree species composition of regenerating stands may have important implications for nutrient cycling dynamics in these forests. Future research should compare the effects on forest floor properties of harvesting and wildfire, more closely examine how harvesting alters organic matter decay patterns in these forest floors and determine whether forest floors from MIXED stands possess different characteristics than those from ASPEN or SPRUCE stands.

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